Serum blood metabolite response and evaluation of select organ weight, histology, and cardiac morphology of beef heifers exposed to a dual corticotropin-releasing hormone and vasopressin challenge following supplementation of zilpaterol hydrochloride[1,2]

J. O. Buntyn,* D. Steffen,† N. C. Burdick Sanchez,‡ S. E. Sieren,* S. J. Jones,* G. E. Erickson,* J. A. Carroll,‡ and T. B. Schmidt*[3]

*Department of Animal Science, University of Nebraska–Lincoln, Lincoln 68583; †School of Veterinary Medicine and Biomedical Sciences, University of Nebraska–Lincoln, Lincoln 68583; and ‡Livestock Issues Research Unit, ARS-USDA, Lubbock, TX 79403

ABSTRACT: The objectives of this study were 1) to determine if supplementation of zilpaterol hydrochloride (ZH) altered select organ weights, histology, and cardiac anatomical features at harvest and 2) to determine if administration of a corticotropin-releasing hormone (CRH) and vasopressin (VP) challenge following 20 d of ZH supplementation altered the blood chemistry profile in cattle. Crossbred heifers (n = 20; 556 ± 7 kg BW) were randomized into 2 treatment groups: 1) control (CON), without ZH, and 2) zilpaterol (ZIL; ZH at 8.33 mg/kg [DM basis] for 20 d). On d 20 of supplementation, heifers were fitted with indwelling jugular catheters. On d 24, starting at 0800 h and continuing until 1600 h, blood samples were collected at 60-min intervals. At 1000 h, heifers received an i.v. bolus of CRH (0.3 μg/kg BW) and VP (1.0 μg/kg BW) to activate the stress axis. Serum was separated and stored at −80°C until analyzed for a large-animal chemistry panel. Following the CRH/VP challenge, heifers were harvested on d 25, 26, and 27 (5, 6, and 7 d after ZH supplementation); BW, HCW, select organ weights, and histology were measured, and a total heart necropsy was performed. A treatment effect ($P \leq 0.02$) was observed for Ca, K, creatinine, alkaline phosphatase, and sorbitol dehydrogenase. Zilpaterol-fed heifers had decreased ($P \leq 0.02$) concentrations of Ca and K and increased concentrations ($P < 0.01$) of creatinine ($P = 0.02$) during the CRH/VP challenge when compared to control heifers. Control heifers had greater ($P \leq 0.05$) alkaline phosphatase and sorbitol dehydrogenase concentrations when compared with ZIL heifers. A treatment × time interaction ($P = 0.02$) was observed for P; concentrations were similar between treatments from −2 to 6 h postchallenge, and 7 h postchallenge CON heifers had decreased P. Liver ($P = 0.06$) and kidney ($P = 0.08$) weights as a percentage of BW tended ($P \leq 0.08$) to be reduced in ZIL heifers. Gross liver weights tended ($P = 0.08$) to be lower in ZIL heifers. Other organ (heart, lung, adrenals) to BW ratios remained similar ($P \geq 0.41$). These data suggest that there are some variations observed between treatments in terms of response to ZH supplementation and the CRH/VP challenge; however, in the environmental conditions of this study, limited variation in blood metabolic responses and organ weights suggests that the supplementation of ZH did not detrimentally alter the physiology of cattle.

Keywords: β-agonist, cattle, heart, pathology, serum profile

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[3]Corresponding author: tschmidt4@unl.edu

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INTRODUCTION

A β-adrenergic agonist can be defined as a phenylethanolamine similar to the natural adrenergic neurotransmitters epinephrine and norepinephrine (Smith, 1998). The binding of β-adrenergic agonists (β-AA) to β-adrenergic receptors forms a complex that activates the G stimulatory (**G_s**) protein. The activation of the Gs protein's α subunit activates the enzyme adenylyl cyclase, which produces cyclic adenosine monophosphate, an intracellular signaling molecule that binds the regulatory subunit of protein kinase A. This binding releases the catalytic subunit allowing for the phosphorylation of intracellular proteins (Mersmann, 1998).

Zilpaterol hydrochloride (**ZH**) is a β_2-adrenergic receptor agonist (**β_2-AA**) that is approved in more than 16 countries (http://www.zilmax.com/food-safety.aspx) for the use in feedlot cattle. Increases in ADG have been observed (Montgomery et al., 2009a) concomitant with slight decreases in DMI during ZH supplementation, resulting in increased feed efficiency (Mersmann, 2002) and an increase in G:F (Avendaño-Reyes et al., 2006). Zilpaterol hydrochloride is associated with an increase in lean tissue deposition in carcass components (addition of 13 and 11 kg in HCW for steers and heifers, respectively; Montgomery et al., 2009a) and a decrease in adipose tissue (Mersmann, 1998, 2002).

Recently, the supplementation of ZH was implicated as a possible cause of reported lameness at harvest (Thomson et al., 2015). A recent report demonstrated an epidemiologic risk of mortality associated with ZH supplementation during the late feeding period but did not investigate the cause of the mortality (Loneragan et al., 2014). Furthermore, limited controlled studies have investigated the impact of ZH supplementation on organ morphology (May et al., 2016). Therefore, the objective of this trial was to evaluate the impact of ZH supplementation on blood chemistry concentrations and internal organ weight and morphology of feedlot heifers at harvest.

MATERIALS AND METHODS

Experimental Design

All experimental procedures were in compliance with the Guide for the Care and Use of Agricultural Animals in Research and Teaching and were approved by the Institutional Animal Care and Use Committee at the University of Nebraska (IACUC number 902).

Twenty ovariectomized English-influenced crossbred heifers (556 ± 7 kg BW) from the University of Nebraska Agricultural Research and Development Center feedlot were selected for this study. Prior to initiation of the study, heifers were haltered and acclimated to being restrained in a tie stall environment and to human contact for a 3-wk period. Heifers were randomly assigned to 1 of 2 treatments: 1) control (**CON**; $n = 10$), fed a finishing diet without ZH (Table 1), and 2) zilpaterol (**ZIL**; $n = 10$), fed the same finishing diet supplemented with ZH (Zilmax; Merck Animal Health, Summit, NJ) at a rate of 8.33 mg/kg BW on a DM basis. For delivery of ZH, 5% of the high-moisture corn in the diet was replaced with 4.9853% fine-ground corn and ZH at 0.0147% calculated to supply ZH at 8.33 mg/kg on a DM basis. Five percent of the CON diet was replaced with fine-ground corn to ensure nutritional similarity between the 2 diets. All supplements were individually mixed into an individual heifer's daily allotment prior to feeding. Heifers were fed once daily at 0800 h for 20 d, followed by a 3-d withdrawal period of ZH. During the 3-d period, the 5% high-moisture corn was returned to both the CON and ZIL diets to replace the 5% ground corn supplement.

Eight days prior to the start of ZH supplementation, heifers were relocated to the University of Nebraska–Lincoln Agricultural Research and Development Center Nutrition Dairy Barn during the month of December 2013. The Nutrition Dairy Barn is a 40-stall barn equipped with individual bunks, automatic waters, and dairy mattresses. Prior to heifers being placed into tie stalls, stalls were randomly assigned to treatment but blocked by treatment group (2 heifers per block) so that no CON heifer shared water with a ZIL heifer. Heifers were maintained in individual tie stalls (1.34 m wide by 1.84 m long) for the duration of the trial. Pine shavings were added on top of the dairy mattress and replaced when needed. On d 20 (last day of ZH supplementation), heifers were removed from tie stalls to obtain a BW (for corticotropin-releasing hormone [**CRH**] and vasopressin [**VP**] dose calculations), fitted with indwelling jugular catheters, and then returned to tie stalls. For the jugular cannulation procedure, heifers were restrained in a working chute, and the neck area was prepped utilizing betadine scrub and ethanol wipes. A small (2 to 3 cm) incision was made in the skin to more easily access the jugular vein. Indwelling jugular catheters, consisting of 30.48 cm of sterile Tygon tubing (AAQ04133; US Plastics, Lima, OH; 1.27 mm i.d. and 2.286 mm o.d.), were inserted into the jugular vein using an 11-gauge by 8.3-cm thin-walled stainless-steel biomedical needle (o.d. = 3 mm). The catheter was stabilized using tag cement (Livestock Identification Tag Cement, The Ruscoe Company, Akron, OH) and a 2.08-cm-wide porous surgical tape around the incision site. The neck region of each heifer was wrapped with vet wrap (Vetrap; 3M Animal Care Products, St. Paul, MN) to ensure stability of the catheterization site. The remaining tub-

Table 1. Composition of finishing diets fed to control (CON) and zilpaterol (ZIL) heifers as a percentage of DM basis during a corticotropin-releasing hormone (0.3 μg/kg BW) and vasopressin (1.0 μg/kg BW) challenge in finishing heifers

	Treatment	
Item	Control	ZIL[1]
Ingredient, %		
High-moisture corn	51.00	51.00
Sweet Bran[2]	40.00	40.00
Wheat straw	5.00	5.00
Fine-ground corn	1.8710	1.8710
Limestone	1.6400	1.6400
Salt	0.3000	0.3000
Tallow	0.1000	0.1000
Beef trace mineral	0.0500	0.0500
Rumensin-90[3]	0.0150	0.0150
Vitamin A-D-E	0.0165	0.0165
Tylan-40[3]	0.0075	0.0075
Supplement[4]		
Fine-ground corn	5.0	4.9853
Zilpaterol hydrochloride	—	0.0147

[1]Heifers received zilpaterol hydrochloride for a 20-d period with a 3-d withdrawal.

[2]Sweet Bran, Cargill Corn Milling, Blair, NE.

[3]Elanco Animal Health; Greenfield, IN.

[4]The control supplement contained only fine-ground corn. The zilpaterol hydrochloride supplement contained (DM basis) 0.0147% Zilmax (Merck Animal Health, Summit, NJ) Type A medicated article and 4.9853% fine-ground corn and supplied zilpaterol hydrochloride supplementation (8.33 mg/kg on a DM basis). Supplement was fed for 20 d.

ing served as the extension portion of the cannula for collection of blood samples (Burdick Sanchez et al., 2013). Environmental temperature and relative humidity data were collected inside the Nutrition Dairy Barn utilizing 4 HOBO U23 Pro v2 temperature/relative humidity data loggers (U23-001, Onset, Bourne, MA). Data from all 4 probes were averaged and compiled to provide an overall environmental temperature and relative humidity. On d 24, the average environmental temperature was 13°C ± 1.2°C, and humidity was 52% ± 3.1%; the average temperature-humidity index on d 24 was 24.8 ± 1.6 within the barn. Prior to d 24, 2 heifers were removed from the trial. One heifer was removed from the trial because of the development of 1 sore underneath the halter, and the other heifer was removed because of the failure of the jugular catheter on d 21; therefore, there were 10 heifers in the CON group and 8 heifers in the ZIL group.

The transportation of cattle results in activation of the hypothalamic-pituitary-adrenal (HPA) axis (Falkenberg et al., 2013); furthermore, the use of the CRH/VP stress model has resulted in a similar endocrine response (Carroll et al., 2007) when compared with an actual relocation event. Therefore, a CRH/VP challenge on d 24 was utilized as a controlled endocrine stressor to produce an endocrine response that would be similar to shipping heifers to the abattoir. On d 24 at 0600 h, all residual feed was removed from bunks, and heifers were not provided daily allotment of feed until 1700 h (completion of the stress challenge). Starting at 0800 h and continuing until 1600 h, 18 mL of blood were collected in Sarstedt tubes containing no additive (Sarstedt Inc., Newton, NC) from each heifer in 60-min intervals. Blood samples were allowed to clot for 30 min at room temperature and then centrifuged at 1,500 × *g* for 20 min at 4°C. Isolated serum was stored at −80°C until analyzed for a large-animal chemistry profile, β-hydroxybutyrate, lactate, and lactate dehydrogenase. At 1000 h (immediately following collection of the blood sample), each heifer received an i.v. bolus of bovine CRH (0.3 μg/kg BW) and arginine VP (1.0 μg/kg BW; Carroll et al., 2007). Following collection of the last blood sample at 1600 h, catheters were removed, and the daily allotment of feed was delivered.

On d 25, 26, and 27, heifers were transported to the University of Nebraska–Lincoln Loeffel Meat Laboratory and harvested under USDA inspection. Heifers were randomly assigned to 1 of the 3 harvest days, with an equal number of CON and ZIL harvested on each day. Individuals collecting organ weights and measurements and conducting histologic examinations were blinded to treatment. At time of evisceration, organ weights were collected. As a means to evaluate all possible changes in visceral tissue, individual (left and right) and gross weights were recorded for kidneys and adrenals. Following weighing of organs, tissue samples of each organ, the pulmonary artery, and the LM were collected, placed into 10% neutral buffered formalin, and stored until processed routinely for histopathology (hematoxylin and eosin staining; Luna, 1968). All tissue slides were evaluated by an American College of Veterinary Pathologists certified pathologist who was blinded to treatment.

Serum Analysis

Serum samples were shipped overnight to the Kansas State Veterinary Diagnostic Laboratory (Manhattan, KS) on dry ice. At the diagnostic laboratory, a large-animal chemistry profile was performed on serum samples utilizing a Roche Cobas C501 chemistry analyzer for serum samples. Serum concentrations of total protein (mg/dL), albumin (g/dL), globulin (g/dL), total calcium (mg/dL), phosphorus (mg/dL), potassium (mmol/L), sodium (mmol/L), sodium potassium ratio, chloride (mmol/L), bicarbonate (mmol/L), anion gap (mmol/L), creatinine (mg/dL), creatine kinase (U/L), aspartate transaminase (**AST**, U/L), alkaline phosphatase

(**ALP**, U/L), γ-glutamyltransferase (**GGT**, U/L), and sorbitol dehydrogenase (**SDH**, U/L) were measured.

Serum β-hydroxybutyrate (βHB) concentrations were determined in duplicate samples by utilizing a βHB assay kit (MAK041; Sigma-Aldrich, St. Louis, MO) in a 96-well format. Plates were incubated at 37°C for 30 min and then read using a plate reader at 450 nm. Concentration of βHB was determined by comparing unknown samples to a standard curve of known βHB concentrations. Data are presented as the concentration in nanograms per microliter.

Serum lactate concentrations were determined in duplicate samples utilizing a lactate assay kit (MAK064; Sigma-Aldrich) in a 96-well format. Plates were incubated at 37°C for 30 min and then read using a plate reader at 570 nm. Concentration of lactate was determined by comparing unknown samples to a standard curve of known lactate concentrations. Data are presented as the concentration in nanograms per microliter.

Serum lactate dehydrogenase (**LDH**) concentrations were determined in duplicate samples by utilizing a LDH activity assay kit (MAK066; Sigma-Aldrich) in a 96-well format. Plates were incubated at 37°C for 2 min, and then an initial absorbance was measured using a plate reader at 450 nm. After the initial reading, subsequent absorbance was measured every 5 min at 450 nm until the most active unknown sample value was greater than the highest standard. The final measurement utilized in calculating the enzyme activity was the penultimate reading or the value before the most active sample was near or exceeded the end of the linear range of the standard curve. Concentration of LDH was determined by comparing unknown samples to a standard curve of known LDH concentrations. Data are presented as the concentration in milliunits per milliliter.

Statistical Analysis

A completely randomized design was utilized in the current study. Data were analyzed using the MIXED (autoregressive covariant structure and ddfm = contain) procedure of SAS specific for repeated measures (SAS Inst. Inc., Cary, NC). For serum blood metabolites, treatment, time, and the treatment by time interaction were included as fixed effects, with heifer within treatment included as the experimental unit. Organ weights were analyzed with treatment included as a fixed effect and heifer within treatment included as the experimental unit. When the main effects were significant, specific treatment comparisons were made using the PDIFF option in SAS, with $P \leq 0.05$ considered significant and $0.05 < P \leq 0.10$ considered a tendency. All data are presented as the least squares mean ± SEM.

RESULTS

A time ($P < 0.001$) effect and treatment × time interaction ($P = 0.001$) were observed for total protein between CON and ZIL heifers (no treatment effect; $P < 0.58$; Table 2). One hour after CRH/VP challenge, total protein decreased (regardless of treatment). Total protein concentrations decreased 1 h after CRH/VP challenge; total protein concentrations 1 h postchallenge were not different ($P \geq 0.58$) compared to prechallenge concentrations. Starting 2 h after CRH/VP challenge, total protein concentrations increased and continued to increase to 8 h after CRH/VP challenge. From 4 to 8 h after CRH/VP challenge, concentrations of total protein were greater ($P < 0.029$) than concentrations observed prior to challenge (−2, −1, and 0 h). For the treatment × time interaction, when individual time points between treatments were evaluated, no time points were different ($P > 0.85$); serum total protein concentrations were 7.60 ± 0.11 and 7.51 ± 0.11 g/dL for CON and ZIL heifers, respectively. There were treatment ($P = 0.02$) and time ($P < 0.001$) effects for serum albumin but no treatment × time interaction ($P = 0.60$). Serum albumin concentrations were 3.64 ± 0.05 g/dL and 3.47 ± 0.05 g dL for CON and ZIL heifers, respectively. A time ($P < 0.001$) effect and a treatment × time ($P = 0.001$) interaction were observed for serum globulin concentrations between CON and ZIL heifers, but no treatment effect was observed ($P < 0.61$). When individual time points were evaluated, no time points were different ($P > 0.87$) between treatment groups, and serum globulin concentrations were 3.95 ± 0.14 and 4.04 ± 0.14 g/dL for CON and ZIL heifers, respectively.

Serum calcium concentrations were affected by treatment ($P = 0.001$) and time ($P < 0.001$); there was no treatment × time interaction ($P = 0.34$; Table 2). Overall, serum calcium concentrations were greater ($P = 0.001$) in CON heifers (9.75 ± 0.07 mg/dL) compared with ZIL heifers (9.49 ± 0.07 mg/dL). For serum phosphorus concentrations, there was no treatment ($P < 0.60$) effect, but a time ($P < 0.001$) effect and treatment × time ($P = 0.02$) interaction were observed. When individual time points were evaluated, no time points were different ($P > 0.80$) between treatment groups. Serum phosphorus concentrations were 5.67 ± 0.19 mg/dL with 5.80 ± 0.19 mg/dL for CON and ZIL heifers, respectively. Treatment ($P = 0.02$) and time ($P < 0.001$) effects were observed for serum potassium, but no treatment × time interaction ($P < 0.13$) was observed. Serum potassium concentrations were 3.90 ± 0.02 and 3.82 ± 0.02 mmol/L for CON and ZIL heifers, respectively. Serum sodium concentrations were not affected by treatment ($P = 0.95$) or treatment × time ($P = 0.53$) interaction. However, there was a time ($P < 0.001$) effect for serum sodium concentrations.

Table 2. Effect of zilpaterol hydrochloride (ZH) supplementation (8.33 mg/kg on a DM basis) on serum metabolites following 20-d ZH supplementation, 3-d withdrawal period, and a corticotropin-releasing hormone and vasopressin challenge administered on d 24

Item	CON[1]	ZIL[1]	SEM	Treatment	Time	Treatment × time
Blood metabolite[2]						
Total protein, g/dL	7.60	7.51	0.11	0.58	<0.001	0.001
Albumin, g/dL	3.64	3.45	0.05	0.02	<0.001	0.60
Globulin, g/dL	3.95	4.04	0.14	0.61	<0.001	0.001
Calcium,[3] mmol/L	9.75	9.49	0.07	0.01	<0.001	0.34
Phosphorus, mg/dL	5.67	5.80	0.19	0.60	<0.001	0.02
Potassium, mmol/L	3.90	3.82	0.02	0.02	<0.001	0.13
Sodium, mmol/L	138.30	138.3	0.24	0.95	<0.001	0.53
Na-K ratio[4]	35.64	36.42	0.25	0.03	<0.001	0.06
Chloride, mmol/L	98.84	97.44	0.19	0.19	0.37	0.26
Bicarbonate, mmol/L	25.73	26.92	0.43	0.06	<0.001	0.88
Anion gap[5]	18.71	17.78	0.30	0.03	<0.001	0.66
Creatinine, mg/dL	1.13	1.40	0.06	0.003	0.02	0.06
CK,[6] U/L	131.80	227.1	49.0	0.17	0.55	0.40
BHB,[7] ng/μL	89.13	83.73	2.35	0.11	<0.001	0.83
Lactate, ng/μL	22.94	20.52	1.02	0.09	<0.001	0.73
LDH,[8] mU/mL	919.93	973.81	45.36	0.39	<0.001	0.41
Liver enzymes						
ALP,[9] U/L	111.6	88.5	8.02	0.05	<0.001	0.88
AST,[10] U/L	156.8	131.1	15.1	0.22	<0.001	1.0
GGT,[11] U/L	48.66	39.00	6.41	0.28	<0.001	0.08
SDH,[12] U/L	64.34	45.04	6.86	0.05	<0.001	0.64

[1]Treatment groups: CON = control (no ZH); ZIL = zilpaterol.

[2]$\beta \geq 0.83$ for serum metabolites.

[3]Total serum calcium.

[4]Serum sodium-potassium ratio.

[5]Calculated serum anion gap.

[6]Serum creatine kinase.

[7]Serum β-hydroxybutyrate.

[8]Serum lactate dehydrogenase.

[9]Serum alkaline phosphatase.

[10]Serum aspartate transaminase.

[11]Serum γ-glutamyltransferase.

[12]Serum sorbitol dehydrogenase.

Serum sodium-potassium ratio was affected by treatment ($P = 0.03$) and time ($P < 0.001$). There was a tendency ($P = 0.06$) for a treatment × time interaction for serum sodium-potassium ratio; when individual time points were evaluated, no time points were different ($P > 0.40$) between treatment groups. Serum sodium-potassium ratios were 35.6 ± 0.25 and 36.4 ± 0.25 for CON and ZIL heifers, respectively. There was no treatment ($P = 0.19$), time ($P = 0.37$), or treatment × time ($P = 0.26$) interaction for serum chloride concentrations. Serum chloride concentrations were 98.8 ± 0.77 and 97.4 ± 0.77 for CON and ZIL heifers, respectively. A time ($P < 0.001$) effect and a tendency ($P = 0.06$) for a treatment difference were observed for serum bicarbonate, but no treatment × time ($P = 0.88$) interaction was observed. Regardless of treatment, serum bicarbonate concentrations fluctuated prior to and after challenge. Serum bicarbonate concentrations were 25.7 ± 0.43 and 26.9 ± 0.43 mmol/L for CON and ZIL heifers, respectively. There were treatment ($P = 0.03$) and time ($P < 0.001$) effects but no treatment × time ($P = 0.66$) interaction for serum anion gap. Regardless of treatment, serum anion gap fluctuated prior to and after challenge. Serum anion gap was 18.7 ± 0.30 and 17.8 ± 0.30 for CON and ZIL heifers, respectively.

Serum creatinine concentrations were affected by treatment ($P = 0.003$) and time ($P = 0.02$), and there was a tendency ($P = 0.06$) for a treatment × time interaction; when individual time points were evaluated, no time points were different ($P > 0.73$) between treatment groups. The time effect was observed starting 1 h after CRH/VP challenge; creatinine concentrations decreased ($P \leq 0.001$) and remained lower than prior to challenge ($P \leq 0.001$). Three hours postchallenge,

creatinine concentrations steadily increased ($P \leq 0.001$) compared to 1 and 2 h postchallenge. This difference continued for the remainder of the postchallenge period (3 to 8 h postchallenge; $P \leq 0.03$) and remained greater than concentrations observed 1 and 2 h after CRH/VP challenge. Overall, creatinine concentrations were lower ($P = 0.003$) in CON heifers (1.13 ± 0.06 mg/dL) when compared with ZIL heifers (1.40 ± 0.06 mg/dL). Creatine kinase concentrations were not affected by treatment ($P = 0.17$) or time ($P = 0.55$), nor was there a treatment × time ($P = 0.40$) interaction. Serum creatine kinase concentrations were 131.8 ± 49 and 227.1 ± 49 U/L for CON and ZIL heifers, respectively.

No treatment effect ($P = 0.11$) or treatment × time interaction ($P = 0.83$) was observed for serum βHB concentrations; a time effect was observed ($P < 0.001$). One hour postchallenge, βHB concentrations were greater ($P < 0.001$) than at all other time points. There were a time effect ($P = 0.001$) and a tendency ($P = 0.09$) for a treatment effect but no treatment × time ($P = 0.72$) interaction for serum lactate concentrations. Overall, serum lactate concentrations were 22.95 ± 1.02 and 20.52 ± 1.02 ng/μL for CON and ZIL heifers, respectively. There was no treatment ($P = 0.39$) or treatment × time ($P = 0.41$) interaction for serum LDH, but there was a time ($P < 0.001$) effect.

Liver Enzymes

There were treatment ($P = 0.05$) and time ($P < 0.001$) effects but no treatment × time interaction ($P = 0.88$) for serum ALP concentrations. Overall, serum concentrations of ALP were greater in CON heifers (111.6 ± 8.02 U/L) compared to ZIL heifers (88.5 ± 8.02 U/L). There was no treatment ($P = 0.22$) or treatment × time ($P = 1.0$) interaction, but there was a time ($P < 0.001$) effect for serum AST. Regardless of treatment, AST concentrations prior to the LPS challenge were similar (−2 0 h; $P \geq 0.22$). One hour after LPS challenge, AST concentrations were greater ($P \leq 0.05$) than concentrations prior to challenge and remained greater until 5 h after LPS challenge. There was no treatment ($P = 0.28$) effect for serum GGT; serum GGT concentrations were 48.66 ± 6.41 and 39.00 ± 6.41 U/L for CON and ZIL heifers, respectively. There were a time effect ($P \leq 0.001$) and a tendency ($P = 0.08$) for a treatment × time interaction; when individual time points were evaluated, no time points were different ($P > 0.64$) between treatment groups. In regard to time effect, prior to the CRH/VP challenge (−2 to 0 h), there was no difference between time points. Starting 1 h postchallenge, GGT concentrations started to increase and were greater ($P \leq 0.008$) than concentrations prior to challenge. This difference between pre- and postchallenge concentrations remained throughout the remainder of the CRH/VP challenge (8 h postchallenge). There were treatment ($P = 0.05$) and time ($P < 0.001$) effects but no treatment × time ($P = 0.64$) interaction for serum SDH. The effect of time was observed 1 h after CRH/VP; 1 to 8 h after the challenge, SDH concentrations were greater than SDH concentrations prior (−2 to 0 h) to the challenge. Overall, SDH concentrations were 64.34 ± 6.86 U/L for ZIL heifers, compared with 45.04 ± 6.86 U/L for CON heifers.

Table 3. Effect of zilpaterol hydrochloride (ZH) supplementation (8.33 mg/kg on a DM basis) on live weights, HCW, and dressing percentage following 20-d ZH supplementation, 3-d withdrawal period, and a corticotropin-releasing hormone and vasopressin challenge administered on d 24

Weights[1]	CON[2]	ZIL[2]	SEM	*P*-value
Initial weight, kg	550	546	11.2	0.79
Final weight, kg	578	575	13.7	0.90
HCW, kg	359	378	9.2	0.17
Dressing percent, %	62.3	65.8	0.5	<0.001

[1]β = 0.66.

[2]Treatment groups: CON = control (no ZH); ZIL = zilpaterol.

Initial BW, final live weight, HCW, and dressing percentage are reported in Table 3. There was no difference in initial live BW ($P = 0.79$), final live BW ($P = 0.90$), or HCW ($P = 0.17$; β = 0.66) between CON and ZIL heifers (Table 3). There was a difference ($P < 0.001$) in dressing percent, with ZIL heifers having a greater dressing percentage compared to CON heifers (65.8% ± 0.5% vs. 62.3% ± 0.5%; Table 3). In terms of select organ weights, there was no effect of treatment for weights for the heart ($P = 0.36$), lungs ($P = 0.30$), total kidneys ($P = 0.11$), left kidney ($P = 0.31$), and left adrenal gland ($P = 0.79$; Table 4). There was a tendency ($P = 0.08$) for liver weights to be greater in CON heifers (7.7 ± 0.10 kg) when compared with ZIL heifers (6.9 ± 0.10 kg; Table 4). Also, there was a tendency ($P = 0.10$) for right adrenal gland weights to be greater in CON heifers (14.3 ± 0.69 g) than in ZIL heifers (12.7 ± 0.69 g; Table 4). There was a treatment difference ($P = 0.03$) for right kidney weights; right kidney weights were greater in CON heifers (595.3 ± 28.9 g) compared with ZIL heifers (504.8 ± 28.9 g; Table 4). As a percentage of BW, there was no treatment effect on heart ($P = 0.45$), lung ($P = 0.41$), and total adrenal gland ($P = 0.42$) weights (Table 5). As a percentage of BW, there was a tendency ($P = 0.06$) for CON heifers to have greater liver and total kidney ($P = 0.08$) weights than ZIL heifers. Liver and kidney weights as a percentage of final BW were 0.65% ± 0.05% vs. 0.61% ± 0.05% for liver and 0.021% ± 0.01% vs. 0.018 ± 0.01% for kidney for CON vs. ZIL, respectively (Table 5). As

Table 4. Effect of zilpaterol hydrochloride (ZH) supplementation (8.33 mg/kg on a DM basis) on major organ weights following 20-d ZH supplementation and 3-d withdrawal period

Organ[1]	CON[2]	ZIL[2]	SEM	*P*-value
Heart, kg	2.64	2.51	0.10	0.36
Liver, kg	7.70	6.90	0.31	0.08
Lung, kg	3.76	3.52	0.17	0.30
Total kidney, g	1,197.24	1,057.5	60.4	0.11
Right kidney, g	595.3	504.8	28.9	0.03
Left kidney, g	602.0	552.7	34.0	0.31
Total adrenal, g	28.8	26.8	1.39	0.32
Right adrenal, g	14.3	12.7	0.69	0.10
Left adrenal, g	14.5	14.2	0.84	0.79

[1]$\beta \geq 0.87$.

[2]Treatment groups: CON = control (no ZH); ZIL = zilpaterol.

Table 5. Effect of zilpaterol hydrochloride (ZH) supplementation (8.33 mg/kg on a DM basis) on major organ weights as a percentage of total BW following 20-d ZH supplementation, 3-d withdrawal period, and a corticotropin-releasing hormone and vasopressin challenge administered on d 24

Organ[1]	CON[2]	ZIL[2]	SEM	*P*-value
Heart	0.46	0.44	0.02	0.45
Liver	1.33	1.19	0.05	0.06
Lung	0.65	0.61	0.03	0.41
Total kidney	0.21	0.18	0.009	0.08
Total adrenal	0.005	0.005	0.0004	0.42

[1]$\beta \geq 0.83$.

[2]Treatment groups: CON = control (no ZH); ZIL = zilpaterol.

a percentage of HCW, there was no difference in heart ($P = 0.45$), lungs (0.41), and total adrenal glands ($P = 0.42$). No treatment differences were observed between CON and ZIL heifers for aortic valve to left atrial ventricular valve diameter ratio ($P = 0.64$), aortic valve to pulmonary valve diameter ratio ($P = 0.16$), aortic valve circumference ($P = 0.98$), or the aortic valve to right ventricular valve diameter ratio ($P = 0.96$; Table 6). Also, there were no differences for interventricular septum thickness ($P = 0.30$), left atrial ventricular valve circumference ($P = 0.64$), left atrial ventricular valve to right atrial ventricular valve diameter ratio ($P = 0.64$), left ventricle free wall thickness ($P = 0.97$), or for the left ventricle plus interventricular septum to right ventricle weight ratio ($P = 0.14$) between treatment groups (Table 6). There was a tendency ($P = 0.07$) for left ventricle plus interventricular septum to total heart weight ratio to be less in CON heifers (0.58 ± 0.05) than in ZIL heifers (0.61 ± 0.05; Table 6). There was no difference in left ventricle free wall thickness to right ventricle free wall thickness ratio ($P = 0.13$), left ventricle thickness to septal thickness ratio ($P = 0.36$), or left ventricle plus septum weight ($P = 0.97$; Table 6). In addition, there was no difference in pulmonary valve to left atrial ventricular valve ratio ($P = 0.47$), pulmonary valve to right atrial ventricular valve ratio ($P = 0.30$), or pulmonary valve circumference ($P = 0.11$; Table 6). There was no difference ($P = 0.94$) for right atrial ventricular valve circumference. There was a tendency ($P = 0.09$) for right ventricle free wall thickness to be thicker in CON heifers (2.11 ± 0.05 cm) than in ZIL heifers (1.99 ± 0.05 cm; Table 6). There was no difference ($P = 0.33$) in right ventricle free wall weight between treatment groups. There was a difference ($P = 0.04$) in right ventricle free wall to interventricular septal thickness ratio, with CON having a greater ratio than ZIL (0.51 ± 0.02 vs. 0.47 ± 0.02, respectively; Table 6). However, there was no difference ($P = 0.72$) for right ventricle to total heart weight ratio between treatments (Table 6). Furthermore, there was no difference ($P = 0.85$) in left ventricle plus interventricular septum as a percentage of BW or right ventricle ($P = 0.30$) as a percentage of BW (Table 7). Gross lesions of hepatitis were seen in 2 CON heifers, and gross nephritis was seen in 1 ZIL heifer (catheter infection observed during the trial). A histologic evaluation of the lungs demonstrated a very mild regional eosinophilic interstitial pneumonia in 1 CON heifer. Pulmonary artery changes were adventitial and consisted of periarterial fibrosis and subacute inflammation and were noted in 2 CON heifers and 1 ZIL heifer. Livers exhibited mild periportal hepatitis in 6 of 10 CON and 7 of 9 ZIL heifers. The 2 CON heifers with gross hepatitis had histologic fibrosis in addition to mild lymphocytic infiltrates. The hepatic lesions were typical mild periportal infiltrates and rare isolated small lymphocytic foci. Renal lesions were mild focal chronic interstitial nephritis consisting of very small, sparse, scattered interstitial aggregates of lymphocytes and plasma cells (6 CON and 6 ZIL heifers). A single foci of lymphocytes was seen in the adrenal medulla of 1 CON heifer. Skeletal muscle and cardiac muscle was histologically normal in all heifers.

DISCUSSION

To our knowledge, an evaluation of complete blood chemistry profiles of cattle supplemented with ZH for a 20-d period in a controlled environment has not been reported. However, similar measurements have been reported in feedlot lambs supplemented with ZH. López-Carlos et al. (2010) reported that ZH supplementation at either 0.10, 0.20, or 0.30 $mg \cdot kg^{-1}$ $BW \cdot d^{-1}$ for the last 42 d of feeding had no impact on serum total protein (López-Carlos et al., 2010). Serum total protein concentrations account for albumin and other globulin frac-

Table 6. Effect of zilpaterol hydrochloride (ZH) supplementation (8.33 mg/kg on a DM basis) on heart measurements following 20-d ZH supplementation, 3-d withdrawal period, and a corticotropin-releasing hormone and vasopressin challenge administered on d 24

Heart parameter[1]	CON[2]	ZIL[2]	SEM	*P*-value
Aortic valve to left atrial ventricular valve diameter ratio	0.71	0.73	0.03	0.64
Aortic valve to pulmonary valve diameter ratio	0.98	1.04	0.03	0.16
Aortic valve circumference, cm	11.61	11.60	0.38	0.98
Aortic valve to right atrial ventricular valve diameter ratio	0.69	0.69	0.04	0.96
Interventricular septum thickness, cm	4.12	4.29	0.12	0.30
Left atrial ventricular valve circumference, cm	16.53	16.13	0.61	0.64
Left atrial ventricular valve to right atrial ventricular valve diameter ratio	0.97	0.95	0.03	0.64
Left ventricle free wall thickness, cm	3.54	3.54	0.08	0.97
Left ventricle + interventricular septum to right ventricle weight ratio	3.28	3.57	0.13	0.14
Left ventricle + interventricular septum/total heart weight, g	0.58	0.61	0.05	0.07
Left ventricle free wall thickness/right ventricle free wall thickness, cm	1.68	1.79	0.05	0.13
Left ventricle thickness/septal thickness, cm	0.86	0.82	0.03	0.36
Left ventricle + septum weight, g	1.53	1.53	0.06	0.97
Pulmonary valve to left atrial ventricular valve ratio	0.72	0.70	0.02	0.47
Pulmonary valve to right atrial ventricular valve ratio	0.70	0.66	0.03	0.30
Pulmonary valve circumference, cm	11.89	11.20	0.30	0.11
Right atrial ventricular valve circumference, cm	17.14	17.08	0.60	0.94
Right ventricle free wall thickness, cm	2.11	1.99	0.05	0.09
Right ventricle free wall weight, g	467.60	436.60	22.40	0.33
Right ventricle free wall to interventricular septal thickness ratio	0.51	0.47	0.02	0.04
Right ventricle to total heart weight ratio	0.18	0.17	0.06	0.72

[1]$\beta \geq 0.91$.

[2]Treatment groups: CON = control (no ZH); ZIL = zilpaterol.

tions and are indicators of overall serum protein status (Evans and Duncan, 2003). Neither supplementation of ZH nor supplementation of ractopamine hydrochloride had an impact on overall serum protein status in feedlot lambs (López-Carlos et al., 2010). Furthermore, feeding clenbuterol to Hereford steers did not impact serum total protein (Ricks et al., 1984). In the current study, ZH supplementation decreased serum total protein in heifers; however, the decrease was minimal (on the basis of reported normal references for cattle). Furthermore, a decrease in total protein would be anticipated because of a decrease of albumin in ZIL-supplemented heifers.

Blood pH affects blood calcium concentrations; decreasing pH will result in the replacement of hydrogen ions with calcium ions (Vagg and Payne, 1970). Furthermore, decreases in albumin can result in decreased calcium concentrations (Russell and Roussel, 2007). In the current study serum calcium concentrations were decreased in ZIL heifers, which also had decreased albumin concentrations. Ricks et al. (1984) reported no difference in blood calcium or phosphorus concentrations of Hereford steers supplemented with clenbuterol in samples collected immediately prior to harvest. Because approximately 99% of mineral storage is in bone, serum circulating concentrations of minerals may be an unreliable measurement on total body mineral status of an animal (Russell and Roussel, 2007). Potassium is the major intracellular cation, which is extensively regulated because of organ interactions (Russell and Roussel, 2007). In the current study, potassium concentrations were decreased in ZIL heifers compared with CON heifers. The decrease in serum potassium concentrations in ZIL heifers could possibly be explained by an increase in lean muscle deposition observed during the utilization of ZH (Vasconcelos et al., 2008). Muscle has been reported to contain 2.43 ± 0.9 mg/g of potassium (Mariam et al., 2004). Serum sodium concentrations correlated to total body sodium because sodium is a major extracellular cation, which is the main contributor to osmotic force and is confined to extracellular fluid (Russell and Roussel, 2007). No differences for serum sodium were expected in the current study as all heifers had ab libitum access to water. The treatment difference observed for the sodium-potassium ratio, with ZIL heifers having greater concentrations than CON heifers, was expected because of the observed differences between treatment groups for potassium.

Chloride is the major anion in extracellular fluid and usually resembles sodium concentrations because of the reabsorption of sodium and chloride together in the kidneys (Russell and Roussel, 2007). In the current study, serum chloride concentrations were not affected by treatment, which was anticipated when compared with serum sodium concentrations. The reference

Table 7. Effect of zilpaterol hydrochloride (ZH) supplementation (8.33 mg/kg on a DM basis) on heart parameter weights as a percentage of total BW following 20-d ZH supplementation

Heart parameters	CON[1]	ZIL[1]	SEM	*P*-value
LV+S BW[2]	0.26	0.27	0.01	0.85
RV BW[3]	0.08	0.08	0.003	0.30

[1]CON = control (no ZH); ZIL = zilpaterol (heifers received zilpaterol hydrochloride for a 20-d period with a 3-d withdrawal).

[2]Left ventricle + interventricular septum as percentage of BW.

[3]Right ventricle as percentage of BW.

range for bicarbonate in cattle is 17 to 29 mmol/L (Kaneko et al., 2008). In the current study, both CON and ZIL heifers had bicarbonate concentrations within the reported reference range. However, bicarbonate tended to be decreased in CON heifers.

The reported reference range for the anion gap for cattle is 13.9 to 20.2 mmol/L (Kaneko et al., 2008). Alterations in the anion gap are commonly utilized to determine acid-base disorders (Gabow et al., 1980), such as during lactic acidosis when the accumulation of the nonchloride anion lactate accounts for an increase in the anion gap (Palmer and Clegg 2017). In the current study, βHB concentrations were within the normal reference range for the anion gap for CON and ZIL heifers. However, CON heifers had a greater anion gap, which could possibly be explained by a tendency for CON heifers to have greater serum lactate. Creatinine concentrations were greater in ZIL heifers but were within the normal reported reference range, 1.2 to 1.9 mg/dL (Kaneko et al., 2008); however, CON heifers had decreased creatinine concentrations compared with normal reported reference ranges. The increase in creatinine was expected due to a 19-kg increase in HCW for ZIL heifers compared with CON heifers. During normal muscle metabolism, creatine is broken down into creatinine, which can be utilized as an indicator of muscle mass in the serum (Russell and Roussel, 2007). Furthermore, serum creatinine is positively correlated with HCW, dressing percentage, and proportion of lean meat in a carcass (Istasse et al., 1990). Cimaterol, a β_2-AA (Signorile et al., 1995), increased plasma creatinine concentrations in Friesian steers during long-term supplementation (Chikhou et al., 1993). Creatine kinase is a key enzyme for cellular energetics (Wallimann et al., 1992). In the current study, there was no difference in creatine kinase between CON and ZIL heifers. Thomson et al. (2015) reported no increase in creatine kinase activity in steers under normal feedlot conditions supplemented with ZH or ractopamine hydrochloride when compared with a control group. Creatine kinase has been reported to increase when steers are transported (Warriss et al., 1995).

β-Hydroxybutyrate is a ketone body produced during the metabolism of NEFA in the liver and can indicate a negative energy balance (Ospina et al., 2010). No difference was observed between treatment groups for βHB in the current study. This lack of a difference is similar to those reported in steers supplemented with clenbuterol (Eisemann et al., 1988). Furthermore, there was also no difference observed for βHB concentrations for steers supplemented with ZH for a 23-d duration (Van Bibber-Krueger et al., 2015). Van Bibber-Krueger et al. (2015) suggested that ZH supplementation in cattle did not alter the metabolization rate of βHB. During anaerobic conditions, lactate is formed by the oxidation of NADH by pyruvate, thus allowing glycolysis to continue (Reece et al., 2015). In the current study, there was a tendency for lactate to be decreased in ZIL-supplemented heifers. Thomson et al. (2015) and Van Bibber-Krueger et al. (2015) reported no difference in lactate concentrations of steers supplemented with ZH. Differences observed between these studies could be explained by the frequency of sampling, as the current study evaluated lactate concentrations during a 10-h CRH/VP challenge following a 3-d ZH withdrawal. Van Bibber-Krueger et al. (2015) reported that ZH supplementation did not alter plasma lactate concentrations, similar to results observed in our trial. These results are in conflict with those reported from previous β-AA supplementation. The inclusion of the β3-AA P-5369 and Q-2636 in milk replacer increased lactate concentrations in calves (Blum and Flueckiger, 1988). Eisemann et al. (1988) observed increased lactate concentrations when clenbuterol was supplemented to steers. Furthermore, arterial cimaterol infusion increased lactate concentrations as cimaterol infusion rates increased (Byrem et al., 1996). Observed differences between the current study and previous studies could be, again, related to the time at which serum samples were collected. In the current study heifers had been withdrawn from ZH supplementation for 3 d, whereas in the aforementioned studies, serum samples were collected during the supplementation period. The glycolytic enzyme LDH catalyzes the conversion of lactate to pyruvic acid (Doornenbal et al., 1988). The reference range for LDH in cows is 692 to 1,445 U/L (Kaneko et al., 2008). In the current study, there was no difference in LDH concentrations between CON and ZIL heifers.

In the current study, serum concentrations for ALP, AST, GGT, and SDH were determined. Alkaline phosphatase catalyzes the cleavage of P*i* from phosphate esters with production of AST originating in the liver of mature animals (Doornenbal et al., 1988). The reference values for AST in cows are 78 to 132 U/L (Kaneko et al., 2008). Ricks et al. (1984) reported no difference in AST in Hereford steers supplemented with clenbuterol. In the current study, AST concentrations were de-

creased in ZIL heifers compared with CON heifers. A possible hypothesis for the decrease in AST concentrations could be related to the decrease in liver weight observed in the current study. It has been reported that in dogs with increased corticosteroid concentrations, AST concentrations are increased because of the formation of a more heat stable isoenzyme of AST (Teske et al., 1986). Previous work from our lab observed a decrease in cortisol in ZIL-supplemented heifers that could explain the increase in AST observed in CON heifers. Aspartate transaminase is an enzyme that catalyzes the transfer of an α-amino group from an AA to an α-keto acid (Doornenbal et al., 1988). In the current study, no difference was observed in AST concentrations. An increase in AST concentrations was observed in Friesian calves administered cimaterol (Chikhou et al., 1993). However, Chikhou et al. (1993) did not observe a difference in liver weights in cimaterol-treated calves, possibly explaining the differences between these studies. γ-Glutamyltransferase is a membrane-bound enzyme that catalyzes the transfer of γ-glutamyl groups from γ-glutamyl peptides to other peptides or AA with a normal reference range of 6.1 to 17.4 U/L (Kaneko et al., 2008). In the current study, there was no difference in GGT between treatment groups; however, both treatment groups were above normal reference ranges. A potential cause of increased GGT in the current study could be associated with the CRH/VP challenge administered. In canines, glucocorticoids have been associated with an increase in GGT concentrations (Shull and Hornbuckle, 1979). Last, SDH concentrations were increased in CON heifers in the current study; however, both treatment groups had elevated SDH concentrations compared with the reference range for cows, which is 4.3 to 15.3 U/L (Kaneko et al., 2008). Feed restriction in llamas resulted in an increase in hepatic lipidosis, which resulted in elevated SDH concentrations (Tornquist et al., 2001). Therefore, the increase in SDH in the current study could potentially be a result of increased lipolysis during the CRH/VP challenge allowing heifers to mount a stress challenge.

Experiments that have evaluated and reported the effects of β-AA on major organs of feedlot cattle are limited. In the current study, there was no difference in final BW or HCW, potentially because of the number of animals on study ($\beta = 0.66$); however, there was a numerical difference in HCW, with ZIL-supplemented heifers having a 19-kg increase compared with CON heifers. Montgomery et al. (2009a) reported an 11-kg increase in HCW with a 20-d supplementation of ZH in heifers. In the current study, there was a positive increase in dressing percentage observed when ZH was supplemented. Montgomery et al. (2009b) reported a dressing percentage increase of 1.2% with feeding ZH. The current study was not designed to evaluate the carcass effects of ZH, but rather a more in-depth analysis of organ parameters with the supplementation of ZH. The increase in dressing percentage of ZIL heifers would indicate a ZH response was initiated.

In the current study, no differences were observed in heart and lung weights between treatment groups. No difference was reported in combined heart and lung weights of beef steers fed ZH for 20 d. However, feeding clenbuterol (β2-AA) to normal mice decreased heart weights by 8% compared with control mice (Sharma et al., 1997). Furthermore, May et al. (2016) reported a tendency for a decrease in heart weights of Holstein steers supplemented with ZH for 20 d but no difference in lung weights between zilpaterol and control steers. A decrease in kidney and liver mass of ZIL-supplemented steers compared with control steers was observed (May et al., 2016). In the current study, there was a difference observed only for right kidney weights, with ZIL-supplemented heifers having lighter right kidney weights; however, overall kidney weights did not differ between treatment groups. Furthermore, there was a tendency for liver weights to be greater in the CON heifers compared with ZIL heifers in the current study. Feeding salbutamol (β2-AA) to pigs has been reported to decrease kidney and liver weights compared with those of control pigs (Hansen et al., 1994).

To our knowledge, data on the effect of β-AA on adrenal weights have not been reported in the literature. Comparing organ weight ratios to BW can result in misleading data because of differences in animal adipose and muscle tissues (Joseph, 1908), as well as the previously reported effect of ZH on HCW (Montgomery et al., 2009a,b). Reported percentages for bovine heart weight ratios to BW are 0.26%, 0.52%, and 0.38% for steers, bulls, and females, respectively (Joseph, 1908). In the current study, heifer heart weight to BW ratios were within this reported range for cattle.

Minimal published anatomic data are available for measurements of the dissection of the bovine heart. The total heart to BW ratios and the left ventricle and septum to right ventricle weight ratios were within the normal range (Leifsson, 2011). The expanded data and reference of a broader range of normal cardiac dimensional anatomy published here will be useful in evaluating finishing cattle affected by or at risk of pulmonary hypertension and chronic right heart failure, which appears to be a growing problem (Neary et al., 2013). The gross lesions of liver fibrosis were observed only in CON heifers, and the kidney lesion was in a heifer with a documented catheter infection and transient sepsis during the study. The mild chronic periportal hepatitis and chronic interstitial nephritis seen equally in both groups are common background lesions seen in cattle in standard production systems. Theses likely reflect

residual inflammation of neonatal infections. The mild adventitial inflammation seen around the pulmonary artery in 2 ZIL and 1 CON heifers is of undetermined etiology but is suspected to be residual scarring and inflammation due to past resolved pulmonary infection. None of these histologic lesions were more prevalent in ZIL heifers and were typical of incidental changes seen in standard commercial cattle. No pathologic changes were observed in these cattle associated with ZH treatment that might suggest a direct link between treatment and reported mortality risk in some production systems.

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