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RUMINANT NUTRITION

Effect of ractopamine hydrochloride on environmental gas emissions, growth performance, and carcass characteristics in feedlot steers

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Abstract

With a growing global population and increased environmental concerns around animal agriculture, it is essential to humanely maximize animal performance and reduce environmental emissions. This study aims to determine the efficacy of feeding ractopamine hydrochloride (**RAC**), an orally active, β_1-adrenergic agonist (**β_1AA**), to feedlot steers in the last 42 d of finishing to reduce ammonia (NH_3) emissions and improve animal performance. A randomized complete block design was used to allocate 112 Angus and crossbred Angus steers (initial body weight [**BW**] = 566.0 ± 10.4 kg) to 8 cattle pen enclosures. Pens (n = 4 per treatment, 14 steers per pen, and 56 steers per treatment) were randomly assigned to one of two treatments: 1) CON; finishing ration containing no RAC, 2) RAC; finishing ration containing 27.3 g/907 kg dry matter (**DM**) basis RAC. Steers were weighed on day –1 and 0 before treatment and day 14, 28, and 42 during treatment. Treatment rations were mixed and delivered daily by masked personnel. Measured emissions included NH_3, nitrous oxide (N_2O), methane (CH_4), hydrogen sulfide (H_2S), and carbon dioxide (CO_2). The primary response variables assessed were emissions standardized by live weight (**LW**) and hot carcass weight (**HCW**). Steers were harvested on day 43 and carcass data were collected on day 43 and 44. Steers fed RAC reduced NH_3 emissions by 17.21% from day 0 to 28 (P = 0.032) and tended to reduce NH_3 from day 0 to 42 by 11.07% (P = 0.070) vs. CON. When standardized for LW, NH_3 was reduced by 23.88% from day 0 to 14 (P = 0.018), 17.80% from day 0 to 28 (P = 0.006), and 12.50% for day 0 to 42 (P = 0.027) in steers fed RAC vs. CON. Steers fed RAC had 14.05% (P = 0.013) lower cumulative NH_3 emissions when standardized by HCW vs. CON. Feeding RAC to Steers reduced H_2S by 29.49% from day 0 to 14 (P = 0.009) and tended to reduce H_2S over day 0 to 28 by 11.14% (P = 0.086) vs. CON. When H_2S emissions were standardized for LW, RAC fed steers had a 28.81% reduction from day 0 to 14 (P = 0.008) vs. CON. From day 0 to 42 the RAC fed steers tended to have a 0.24 kg/d greater average daily gain (**ADG**) (P = 0.066) and tended to eat 4.27% less (P = 0.069) on a DM basis vs. CON. The RAC fed steers had a 19.95% greater gain to feed ratio (**G:F**) compared to CON (P = 0.012). Steers fed RAC had an average of 12.52 kg greater HCW (P = 0.006) and an increase of 1.93 percentage units in dressing percent (**DP**) (P = 0.004) vs. CON. Ractopamine is an effective medicated feed additive for reducing NH_3 and improving end product performance through HCW yields.

Key words: ammonia emissions, beef cattle, beta agonist, feedlot cattle, hot carcass weight, ractopamine hydrochloride

Abbreviations

ADG	average daily gain
BW	body weight
CPE	cattle pen enclosure
DE	digestible energy
DM	dry matter
DMI	dry matter intake
DP	dressing percent
FDA	U.S. Food and Drug Administration
G:F	gain to feed ratio
HCW	hot carcass weight
KPH	kidney, pelvic and heart fat
LOQ	limit of quantification
LW	live weight
ME	metabolizable energy
MW	molecular weight
NEm	net energy for maintenance
NEg	net energy for gain
$PM_{2.5}$	particles with aerodynamic diameter less than or equal to 2.5 µm
ppb	parts per billion
ppm	parts per million
PA	proximate analysis
QG	quality grade
RAC	ractopamine hydrochloride
REA	ribeye area
RPM	rotation per minute
YG	yield grade
βAA	beta-adrenergic agonist
β_1AA	β1-adrenergic agonist
β_2AA	β2-adrenergic agonist

Introduction

By the year 2050, the Food and Agriculture Organization of the United Nations projects the world population will grow to 9 billion people and there will be a corresponding 75% increase in demand for animal protein (Alexandratos and Bruinsma, 2012). To satisfy increased demands for animal protein, it is essential to increase animal production while minimizing resources used for production. The United States is the current world leader in beef production, producing 17% of the world's beef with 6% of the global herd (UN FAOSTAT, 2018). Efficiencies in the United States must be mimicked around the world in order to meet the growing global demand for animal protein. With increased animal production comes a potential for increases in emissions to land and air which pose a threat to the environment. Air emissions of primary concern in animal agriculture are the greenhouse gasses methane (CH_4) and nitrous oxide (N_2O) as well as criteria pollutants ammonia (NH_3) and hydrogen sulfide (H_2S; NRC, 2003). It is critical to monitor and minimize these criteria air pollutants when working to improve animal production so as not to have unintended effects on nitrogen deposition and human health concerns.

The criteria pollutant, NH_3, is of particular interest when considering emission reductions from beef production. Ammonia is produced when urine combines with feces and the urea in urine is rapidly converted by urease in the feces to form NH_3, and volatized (Bouwman et al., 1997). Cattle retain a portion of nitrogen they consume from feed, but approximately 70% to 90% of nitrogen is excreted in the feces and urine (Cole et al., 2008). Ammonia is not only a noxious gas but is also a precursor to the formation of ammonium sulfate, ammonium bisulfate, or ammonium nitrate which are all classified as $PM_{2.5}$ (fine particulate matter with aerodynamic diameter of less than or equal to 2.5 µm; USEPA, 2004). When these aerosols are inhaled, they can carry pathogens that infiltrate the alveoli of the lungs and enter the blood stream (Aneja et al., 2008). Continued exposure can cause illness and respiratory disease especially in people with conditions such as asthma (Samet et al., 2000).

To date, there has been one U.S. Food and Drug Administration (**FDA**) approved product for the reduction of NH_3 emissions from beef cattle. Experior (lubabegron; NADA 141–508; Elanco Animal Health, Greenfield, IN; Elanco, 2018) is the first Type A medicated article with a gas emission label claim indicating a reduction in NH_3 emission per unit of live weight (**LW**) or hot carcass weight (**HCW**) when fed to cattle during the last 14–91 d prior to slaughter (Elanco, 2018). Experior is a beta-adrenergic agonist (**βAA**), a class of compounds commonly fed to cattle for their ability to increase the accumulation of skeletal muscle protein, improve growth rate and feed conversion efficiency (NRC, 1988).

Beta agonists bind to receptors on fat cells and redirect and reduce the metabolism of fat allowing for more protein accretion (NRC, 1988). More nitrogen is utilized during muscle accretion which, in theory, will lead to a reduction in N excreted in urine and a subsequent reduction in NH_3 emissions. Concurrently, βAA bind to receptors on muscle and fat cells and repartition nutrients to increase lean muscle tissue and/or increase lipolysis which ultimately results in a leaner, more efficient animal. The reduction in fat deposition and increase in lean muscle accretion reduces the energy needed from feed to increase weight gain, thus leading to improved feed efficiency in cattle (NRC, 1988; Johnson et al., 2014).

Ractopamine hydrochloride (**RAC**), an orally active, β_1-adrenergic agonist (**β_1AA**) has a similar mode of action to Experior (Elanco, 2018, Johnson et al., 2014). Currently, there are three FDA-approved RAC products for beef cattle: Optaflexx 45 (NADA 141–221; Elanco Animal Health, Greenfield, IN), generic Actogain 45 (ANADA 200–548; Zoetis, Parsippany, NJ), and generic Optigrid 45 (ANADA 200–679; Huvepharma, St. Joseph, MO). This study used generic Actogain 45 (Zoetis) for the RAC treatment. It was hypothesized that RAC would reduce NH_3 emissions when fed the last 42 d prior to slaughter.

The objective of this study was to determine the effect of RAC on gaseous emissions, growth performance, and carcass characteristics when fed via complete feed (27.3 g/907 kg, dry matter [**DM**] basis) in beef steers fed in confinement during the last 42 d on feed.

Materials and Methods

Study location and standards

This study was conducted at the University of California, Davis, Feedlot Teaching and Research Facility with the approval of the research protocol number 21405 by the Institutional Animal Care and Use Committee and in accordance with the following U.S. standard and international guidance: Good Clinical Practice standards, FDA Guidance No. 85 (FDA, 2001).

Experimental design, treatments, and cattle

A randomized compete block design was utilized to evaluate the effect of RAC on gaseous emissions, growth performance, and carcass characteristics over a 42-d period using 112 Angus and crossbred Angus steers (Initial BW = 566.0 ± 10.4 kg) housed in 8 cattle pen enclosures (**CPE**). Fourteen animals were housed

in each CPE, and treatment rations were fed once daily. Each CPE was randomly assigned to one of two treatments (n = 4): 1) CON; finishing ration containing no RAC, 2) RAC; finishing ration containing 27.3 g/907 kg DM basis RAC. Prior to study initiation, treatments were randomly assigned a masked color code of either green or red. All CPEs and other related treatment items such as pre-mix containers, Type B mixers, and so on were identified with color-coded tape (green or red) to ensure masking and to differentiate blinded treatments. Emissions measured included the following: NH_3, N_2O, CH_4, H_2S, and carbon dioxide (CO_2). The primary response variables assessed were the ratios of these gaseous emissions to final body weights (**BW**) or LW and HCW. All personnel involved in daily feeding activities and data collection were masked to treatments to prevent any bias during the trial phase. Only two personnel who weighed out the pre-mix were unmasked (knew which color represented RAC treatment and which color was control). The unmasked personnel were not involved in any other feeding procedure or data collection.

Study timeline and body weight measurements

Steers were sourced by Johnson Research from Boise Valley Feeders in Parma, ID. Growth-promoting implants were removed via excision by a veterinarian at Johnson Research 28 d prior to day 0 (beginning of treatment phase). Steers were transported 901 km from Parma, ID, to the UC Davis feedlot in Davis, CA, on day –15 with arrival on day –14. Steers arrived at the UC Davis Feedlot on day –14 where they were weighed and a veterinarian verified steers were healthy and that there were no growth-promoting implants present. Steers were housed in outdoor pens with shade during the 14-d acclimation period (day –14 through day –1). Steers were observed daily for any abnormal health events. Upon arrival, steers were fed a transition ration top-dressed with wheat hay from day –14 to day –11. Steers were then transitioned to a finishing ration on day –10 and fed the finishing ration throughout the rest of acclimation and treatment phase (day –1 to day 42; Tables 1 and 2). On day –1, BW were recorded and used to randomize steers to their respective CPE. Day –1 feed refusals were collected and processed to determine the dry matter intake (**DMI**) from the acclimation phase. This information was used to determine an appropriate feed call for day 0. A licensed veterinarian was present on day –1 to ensure steers were eligible for enrollment which was determined based upon BW, health, and implant status (no implant present and healed ear) which was verified via ear palpation by a UC Davis veterinarian. On day 0, cattle were weighed and moved into their assigned CPE based on the randomization for the duration of the 42-d treatment period. Treatment rations were fed starting on day 0 through day 42. Daily feed samples were collected for weekly proximate analysis (**PA**) and RAC concentration analysis. Emission measurements began when cattle entered CPE on day 0 and concluded when they were removed on day 42.

BW and feed refusals were collected on day 0, 14, 28, and 42. On each weigh day, residual feed was collected from all pens prior to weighing steers. Steers were weighed in the morning before feed was administered to pens. An Avery Weigh Tronix 640N (Fairmont, MN) chute was used to weigh steers. Prior to weighing steers, the chute was verified within a 2% error range using certified check weights for a low end of 181 kg and high end of 680 kg. On day 42, cattle were transported 1008 km to Washington Beef LLC (Toppenish, WA) for slaughter and carcass data collection.

Mixer wagon validation, diet formulation, feed mixing, and feeding

A mixer wagon validation was conducted on the Roto-Mix feed wagon (Model 274-12B; Dodge City, KS) prior to the beginning of the current study to ensure adequate mixing of RAC with varying amounts of completed feed resulted in a homogenous ration with the inclusion of RAC at appropriate levels. A 227 kg and a 680 kg batch of the RAC ration was mixed and primary and backup samples were taken at 10% intervals of the ration being disbursed to be assayed for DM and RAC concentration. To ensure no residual RAC feed remained in the mixer, a 227 kg CON batch was mixed after flushing the Roto-mix with water. The CON batch was sampled in the same manner as the RAC batches previously discussed and were analyzed for DM and RAC concentration. The RAC batches all came back within acceptable ranges for RAC concentration and the CON batch showed no signs of carry-over of RAC treatment.

Diets were mixed on site daily at the University of California, Davis Feed Mill. The study ration composition is represented in Tables 1 and 2. The complete diet was mixed for 8 min, followed by mixing of type B for 3 min. The complete diet was formulated to meet all nutrient requirements of growing beef cattle (NRC, 2016).

For daily feedings in the CPE, the complete diet was added to the Roto-Mix feed wagon according to the daily feed call for each treatment. After masked study personnel made the feed

Table 1. Composition of study ration on an as-fed basis (%)

Ingredient[1]	Ration
Dry Rolled Corn, %	76.88
Molasses, %	7.39
Wheat Hay, %	12.07
Limestone, %	1.57
Urea, %	1.6
Magnesium, %	0.17
Trace Mineral Salt[2], %	0.32

[1]A ground corn carrier including a 0.45 kg intermediate premix with or without ractopamine was included at 2% (as fed basis) while the remaining 98% (as fed basis) consisted of the finisher ration.
[2]Contains: salt (96.175%), manganous oxide (1.666%, 9996.156 mg/kg), vegetable oil (1%), zinc oxide (0.8335%, 6001.291 mg/kg), copper sulfate (0.16%, 407.194 mg/kg), ethylene diamine dihydroiodide (0.0255%), sodium selenite (0.1475%, 22.123 mg/kg), and 202.728 mg/kg iodine (A. L. Gilbert Company, Oakdale, CA).

Table 2. Analyzed chemical composition of study ration on a DM basis

Ingredient	Ration
DM, %	82.0 ± 1.2
Crude protein, %	12.2 ± 0.8
Crude fiber, %	10.7 ± 3.8
Total digestible nutrients, %	80.9 ± 6.8
Crude fat, %	2.58 ± 0.36
Ash, %	4.70 ± 0.32
DE[1], Mcal/kg	3.57 ± 0.31
ME[2], Mcal/kg	2.93 ± 0.24
NE_m[3], Mcal/kg	1.96 ± 0.20
NE_g[4], Mcal/kg	1.32 ± 0.18

[1]Digestible energy.
[2]Metabolizable energy.
[3]Net energy for maintenance.
[4]Net energy for gain.

calls each morning, they gave the color-coded (CON = green; RAC = red) feed call totals (n = 2, CON or RAC) to the unmasked study personnel who then went to a locked room where RAC was stored to create the intermediate pre-mix that did or did not contain RAC (CON or RAC). An intermediate premix was created by designated unmasked study personnel daily for RAC and CON. The amount of Type A ractopamine hydrochloride (99.9 g/kg RAC, Actogain 45, Zoetis) included in the intermediate premix was determined for each treatment based on diet DM, daily feed call, and desired dose (CON, RAC = 27.3 g/907 kg DM Basis) and was mixed with 0.45 kg of ground corn in a masked color-coded container. To make sure masked personnel were unable to discern weight differences, an additional amount of ground corn was added to the CON 0.45 kg ground corn premix to match the amount of RAC added to the 0.45 kg ground corn to create the intermediate premix. The color-coded intermediate premixes (green or red containing CON or RAC) were then given to masked study personnel who then blended the color-coded intermediate premixes with the remainder of the ground corn carrier (2% of the batch size, as fed basis) in a Ryobi RMX001 mixer (Anderson, SC) for 2 min to create a Type B medicated/nonmedicated article. For each treatment, the Type B was then added to the complete feed diet and mixed in the Roto-Mix feed wagon for 5 min before being fed out to the appropriate CPE by masked study personnel. Order of feed delivery (by treatment) were randomized prior to study start and maintained throughout study duration.

Steers were fed ad libitum throughout the trial. Bunks were assessed daily, and feed calls were made with the goal of 4.5 and 9 kg per CPE residual feed to ensure animals had ad libitum access to feed.

CPE were fed in the same randomized order every day by masked study personnel. The mixer wagon was flushed with water between batches and after the final batch to ensure there was no cross contamination between CON and RAC batches. Steers were fed once daily at 0700.

Feed sampling

Starting on day 0, two feed samples, a primary and backup (approximately 2.3 kg each), were taken daily from each CPE. Feed samples were factored into the feed consumption data to account for the loss of feed. The daily primary feed samples were then mixed into a composite by color-coded treatment using a Ryobi RMX001 mixer. Samples were mixed for approximately 2 min before daily primary and backup composite samples (approximately 2.3 each) kg were taken from each treatment with the remainder discarded. At the end of each 7-d period, the seven backup daily composite samples were retained frozen at –20 °C and the seven primary daily composite samples for each treatment were combined and mixed for approximately 2 min before being subsampled to obtain three weekly composite samples (approximately 2.3 kg) for each treatment (PA, ractopamine concentration, and backup). One weekly composite feed sample for each treatment was sent to Servi-Tech Laboratories (Amarillo, TX) for PA to obtain percent DM, crude protein, crude fat, crude fiber, total digestible nutrients, and ash. Weekly composite feed sample for each treatment was sent to Zoetis (Kalamazoo, MI) to be tested for percent recovery of RAC to ensure drug inclusion was within an acceptable range of the target. The permitted analytical variation for RAC samples was 75% to 125% of the target concentration (Covance Method, 2012) with a coefficient of variation ≤15% between all the samples in a given treatment. The control samples were acceptable if at or below the limit of quantification (**LOQ**; ≤2.3 g/907 kg or ≤2.5 ppm as-is). If the weekly composite feed sample for RAC percent recovery fell outside of the target concentration range, the backup weekly composite sample was then tested and if the backup weekly composite failed, then the seven daily composite samples were tested to determine the day or days where the issue occurred.

Feed samples were frozen until shipped for analysis. A temperature log was recorded daily to ensure proper storage temperatures for feed samples. Backup samples for both daily and weekly composites were retained on-site until results from laboratories were obtained.

DM obtained from weekly composite PA were used to establish the DM composition of the complete feed. Feed refusals were weighed in the morning on each weigh day, and a sample was taken from each CPE for DM analysis. This was used to determine the DM remaining in the pen which was subtracted from the total DM fed during that period to establish a DMI for each period.

General health observations

Following arrival to the feedlot facility through harvest, steers were monitored daily until the steer was removed from or completed the study, including up to harvest. An abnormal health event was considered any observation in steers that was unfavorable and unintended and occurred after the start of treatment, whether or not considered to be product related. Any abnormal health event that was observed was recorded and a veterinarian was called to determine treatment until the abnormal health event was resolved or the steer was removed from study.

Gas emission data and collection

Each CPE was a 22.0 m × 11.3 m hoop house shaped building with a maximum height of 6 m. CPE were constructed with a steel frame (11 m Legend Series Cover-All Building, Saskatoon, Saskatchewan, Canada; Figure 1) which was covered with a double stacked Dura-Weave cover (Intertape Polymer Group, Montreal, Quebec, Canada). Each CPE contained 185 m^2 of soil surface, simulating a dirt feedlot floor, and 9.1 m of linear bunk space. Each CPE was equipped with a 4.88 m × 1.22 m cooling pad on the east side to allow ambient air inflow and evaporative cooling. Figure 1 shows both the interior of a CPE with cattle present and the exterior of the CPE from the entrance side. Two fans with ventilation openings on the west side provided air outflow. Fan speed and cooling pad operation were controllable inside the CPE. Fan rotations per minute (**RPM**) were monitored constantly by two RPM sensors (Monarch Instruments, Amherst, NH) mounted on every fan unit in each CPE. Air flow rates through each CPE were measured before the study started. The RPM of each fan and the static differential pressure between inside and outside CPE are recorded to monitor the changes in CPE flow rate. Long-term drifts in CPE flow rates throughout the 42-d period of the study were less than 4% and the daily changes in CPE flow rates were less than 1%. The fans created a negative pressure by venting air out of the corral providing directional airflow from east to west in each CPE.

A TEI 55i Direct Methane Non-Methane Hydrocarbon analyzer (Thermo Environmental Instruments, Waltham, MA; the same for all TEI analyzers) was used to measure CH_4 and non-methane total hydrocarbons, TEI 450i measured H_2S and sulfur dioxide (SO_2). A TEI 46i monitored N_2O. A TEI 17i measured nitric oxide (NO), nitrogen dioxide (NO_2), and NH_3. A TEI 410i measured CO_2. As a back-up, a photoacoustic field gas-monitor, an INNOVA

model 1412 (INNOVA, AirTech Instruments, Ballerup, Denmark), continuously measured CH_4, CO_2, NH_3, and N_2O. Data collected using the INNOVA analyzer were not used as all TEI analyzers performed adequately during the study. Gas measurements, in 20 min intervals, were obtained in sequential order starting with the inlet, followed by the eight outlet locations of each respective CPE. The INNOVA 1412, TEI 55C, TEI 450i, TEI 46i, TEI 17i, and TEI 410i continuously measured gas concentrations at 1-min intervals. Detection limits for all analyzers are given in Table 3. Although additional gas emission data were measured by the analyzer systems, only NH_3, CH_4, CO_2, and H_2S were analyzed. Zero check with ultra-zero air and span check with standard calibration gases were performed weekly. If any results of the zero and span checks exceeded the preset thresholds, the corresponding gas analyzer was calibrated to correct long-term drifts. Only TEI 46i that measured N_2O gas were calibrated frequently due to its drifting. Since the difference between inlet and outlet concentrations was nondetectable for N_2O, results of N_2O measurements are not presented.

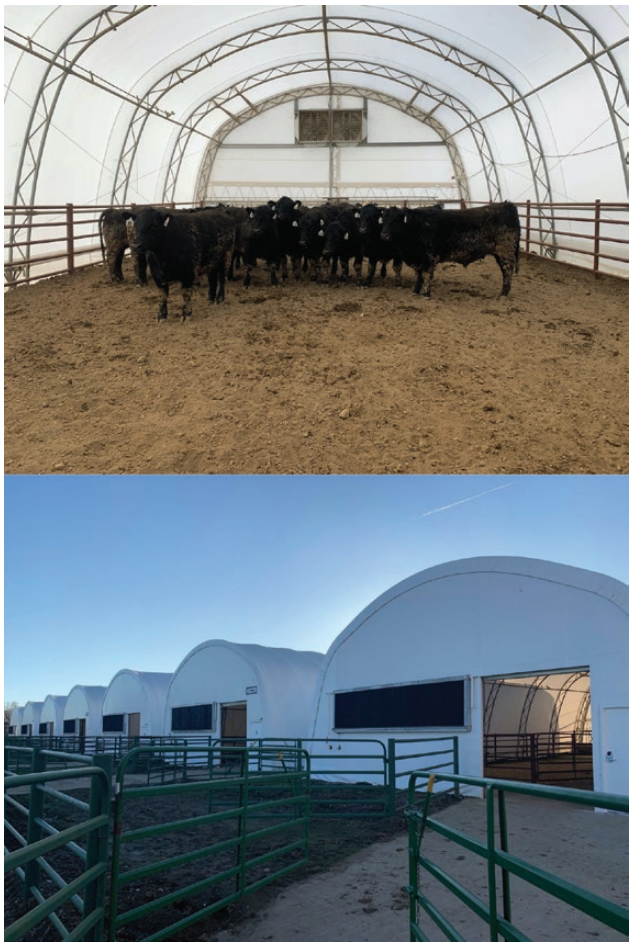

Figure 1. Interior and exterior of CPE.

The air sampling equipment and data logging computers were located centrally in an air-conditioned modular building. For each CPE, 48.8 m of teflon tubing (9.5 mm outer diameter) was used to connect the CPE sampling location to the gas analyzers, which were kept in a trailer at or below 22.22 °C. Net emissions were calculated as the concentration difference between the air outlet and inlet and multiplied by the ventilation rate. Data corresponding to short time interruptions in which the CPE was opened for feeding or entry had 5 min truncated after closing for calculation of emission fluxes. Data were not excluded for entrance into CPE through the access door for health observations as there was a negligible effect on emissions from the opening and closing of this door.

Ambient temperature (°C) and relative humidity (%) were measured continuously (10 min intervals) within each CPE using a temperature/humidity transmitter (Dwyer Instruments, Inc., Michigan City, IN). Meteorological measurements were obtained outside the CPE using an automatic weather station (Novalynx, Model 110-WS-16, Auburn, CA), which was centrally located at the east end of the CPE. Outside measures were recorded in 15 min intervals and include ambient temperature (°C), black globe temperature, relative humidity (%), and wind velocity (m/s).

This sampling procedure was continuous, resulting in six to eight sampling periods per day per CPE. These sampling periods were averaged by CPE and gas to provide a mean daily emission rate. The average daily emission rates were summed by CPE for a particular gas to provide cumulative gas emissions for the treatment phase.

Accumulation of excreta in the CPE began on day 0 when steers were allocated to the 8 CPE. Excreta in each CPE were left inside for the duration of a study.

Emissions calculations

Emissions were measured as the ratio of analyte gas volume to total air volume and were reported in parts per million (**ppm**) for CH_4, CO_2, and N_2O, and parts per billion (**ppb**) for NH_3 and H_2S. To calculate emission rates for each 15-min sampling period, the concentration of analyte gas in the sample was converted to g/min using the molar gas volume in the following equation (equation 1):

Table 3. Gas analyzers and their respective detection limits and detection ranges

Gas analyzer	Gases	Detection limits	Detection ranges
TEI 55i Direct Methane Non-Methane Hydrocarbon analyzer	CH_4	0.033 µg/L	0–67 µg/L
TEI 410i CO_2 Gas Analyzer	CO_2	0.37 µg/L	0–1.83 mg/L
TEI 450i SO_2/H_2S analyzer	SO_2	0.004 µg/L	0–27 µg/L
	H_2S	0.002 µg/L	0–14 µg/L
TEI 46i N_2O analyzer	N_2O	0.037 µg/L	0–37 µg/L
TEI 17i NH_3 analyzer	NH_3	0.001 µg/L	0–14 µg/L
Innova 1412 photo-acoustic multi-gas analyzer	CO_2	2.7 µg/L	0–27 mg/L
	CH_4	0.27 µg/L	0–2.7 mg/L
	NH_3	0.71 µg/L	0–7.1 mg/L
	N_2O	0.055 µg/L	0–0.55 mg/L

$$Total\ Flux\ \left(\frac{g}{min}\right) = \frac{\frac{(Gas\ ppm - Incoming\ ppm) \times Air\ Flow\ \frac{m^3}{min} \times 1{,}000\ \frac{L}{m^3}}{\frac{V_s\ \frac{L}{mol}}{273.15\ K} \times (Temp\ ^{\circ}C + 273.15)}}{1{,}000{,}000} \times MW\ \frac{g}{mol}$$

where Gas ppm (or ppb) = gas concentration in the CPE air sample; incoming ppm (or ppb) = gas concentration in ambient air; Airflow (m^3/min) = airflow rate through the CPE; V_s (L/mol) = molar volume of a gas at constant temperature and pressure (V_s (L/mol) = 22.4 at ambient conditions); MW (g/mol); = molecular weight (**MW** = 16.04 g for CH_4, 44.01 g for N_2O, 44.01 g for CO_2, 34.08 g for H_2S, and 17.03 g for NH_3); and temperature (°C) converted to kelvin (K).

In order to standardize calculated values to g, the denominator was 1,000 times greater for variables measured in ppb compared to variables measured in ppm (1 million for ppm, 1 billion for ppb).

The concentration of analyte gas in ambient air was subtracted from the concentration of analyte gas in samples from each CPE to adjust for baseline values and supply the net amount contributed by the CPE (equation 1). The net concentration was multiplied by the CPE airflow rate to yield the net emission rate (g/min). The emission rates were then averaged over all sampling periods occurring within defined 24-h periods to produce the daily emission rate (g/min) for individual gases from a CPE. Finally, daily emissions per steer were determined by multiplying the CPE average g/min emission rate by 1,440 min to convert to cumulative daily emissions. Cumulative daily emissions per CPE were then divided by the number of cattle present in the CPE on that d in order to account for any removals during the treatment phase. The resulting daily emission rates (g per steer) were summed over each interim BW measurement period (day, 0 to 14, 0 to 28,) and over the entire 42-d period to provide cumulative gas emissions, cumulative gas emissions/kg BW, and cumulative gas emissions/kg HCW on a per steer basis.

Slaughter and carcass data collection

On day 43, steers were shipped (1,008 km) from the UC Davis Feedlot in Davis, CA to Washington Beef (USDA establishment number: EST. 235) in Toppenish, WA for harvest and carcass data collection. Steers were observed for ambulatory status and health by a veterinarian prior to shipping and before slaughter. Steers were not fed upon arrival at Washington beef, but had ad libitum access to water. Cattle were harvested in accordance with USDA requirements and standard site procedures. HCW was recorded on day 43. Kidney, pelvic, and heart fat (**KPH**) were evaluated on hot carcasses (d 43); KPH was removed from hot carcasses; therefore, the collection could not be done on a chilled carcass. Personnel from Johnson Research, LLC. (Parma, Idaho) and University of California, Davis collected carcass data.

Chilled carcasses were evaluated on day 44. Ribeye area (**REA**) was measured at the 12th rib interface on blotting paper then traced on a double matted acetate paper while at the harvest facility and two trained personnel used a grid to determine the appropriate REA. Fat thickness was measured at a point ¾ of the length of the longissimus muscle from the chine bone at the 12th rib. Marbling was expressed as a percentage or degree of intramuscular fat deposition converted to a standard number score. Scores were reported to the nearest 10 degrees of marbling. Lean and skeletal maturity were expressed as a percent or degree of maturity converted to a standard number score. Scores were reported to the nearest 10 degrees of maturity. Skeletal maturity and lean maturity were compiled for the determination of the overall maturity. Marbling score, overall maturity, and any associated comments (e.g., dark cutters) were compiled for the determination of USDA quality grades (**QG**). The primary QG for beef used for this study were: Prime, Choice, Select, and Standard with additional subdivisions of high (+), average (0), and low (-) for improved classification of carcass quality. Dressing percent (**DP**) was calculated from the following: HCW divided by the end-of-study individual BW multiplied by 100, pencil shrink of 4% was not applied to the DP. The USDA Yield Grade (**YG**) was calculated from the following formula (equation 2; USDA, 2005):

$$\begin{aligned} Yield\ Grade = {} & 2.5 + [2.5 \times fat\ thickness\ (cm)] \\ & + [0.20 \times KPH\ (\%)] + \\ & [0.00138 \times HCW\ (kg)] - [0.32 \times REA\ (cm^2)] \end{aligned}$$

Statistical analysis

Randomization of eligible steers to pens and treatments was performed according to a randomized complete block design. Blocking was based on day –1 BW and CPE location. There was a total of 8 CPE, which were the experimental unit. All statistical analyses of data utilized SAS Release 9.4 (SAS Institute, Cary, NC). Treatment contrasts were assessed using two-sided tests at the 5% level of significance ($P \leq 0.05$), with trends determined at $0.05 < P \leq 0.10$.

Gas Emissions, average daily gain (**ADG**), gain to feed ratio (**G:F**), and average daily feed intake were analyzed for each time period using a general linear mixed model (PROC MIXED) with the fixed effect of treatment and the random effects of block. Pen averages were computed for HCW, USDA calculated YG, marbling score, skeletal maturity, lean maturity, DP (calculated as 100 × HCW/final individual steer weight), 12th rib fat thickness, REA, and KPH. The pen averages were then analyzed using a general linear mixed model (PROC MIXED) with the fixed effect of treatment, and random effect of block. The minimum Akaike's Information Corrected Criterion was used to determine whether an equal or unequal variance for treatment groups was needed (Group = Treatment option). A priori contrasts were conducted to compare CON to RAC. For USDA QG, the percentage of carcasses that graded prime, choice, or select were each analyzed separately using a generalized linear mixed model (PROC GLIMMIX) with binomial error distribution and logit link function. The model included the fixed effects of treatment and random effects of block. When the generalized linear mixed model did not converge, Fisher's exact test was applied. A priori contrasts were conducted to compare CON to RAC.

Results

Feed sampling

Complete feed samples of both CON and RAC treatments were within the acceptable range for each week of the study. Each week, CON treatment with a target of 0 g/907 kg (DM basis) RAC fell below the LOQ (≤2.3 g/907 kg or ≤2.5 ppm as-is). The week 5 RAC sample initially fell out of the acceptable range; however, after further analysis, the backup weekly composite sample and all seven daily composites fell within the acceptable range. The weekly feed samples for RAC were within the acceptable range with a mean recovery of 24.3 ± 2.4 g/907 kg (DM basis) RAC of the desired 27.3 g/907 kg (DM basis, Table 4).

Table 4. Summary of the percent ractopamine hydrochloride recovery of each treatment

	Treatment[1]	
Item	CON	RAC
Target, g/907 kg (100% DM)	0	27.3
Actual, g/907 kg (100% DM)	<LOQ	24.3 ± 2.4
Mean Actual Recovery[3], %	<LOQ	89.0 ± 9.0

[1]Treatments: CON = finishing ration containing 0 g/907 kg RAC DM basis (Control); RAC = finishing ration containing 27.3 g/907 kg RAC DM basis (Ractopamine).
[2]Acceptable range = 75–125%.

Animal health

There were three steers treated for foot rot during the study, one in CON and two in RAC-fed pens. The CON steer remained in the study after effective treatment while the two RAC steers were removed from study upon recommendation of the study veterinarian as symptoms did not improve. There was one mortality from bloat during the study in the CON group. One steer in the RAC fed group was seen by the study veterinarian for lethargic behavior but recovered the following day with no additional symptoms. Data for gas emissions, ADG, G:F, and DMI were included for removed and deceased animals until point of removal. There was one exception where the BW data of one steer in the RAC-fed group was removed from BW and ADG data due to the steer (Steer 32, RAC) being condemned at harvest and diagnosed with sepsis. Blood tests and general health exam from study veterinarian came back inconclusive so the steer remained on study.

Gas emissions

Ractopamine had no effect ($P \geq 0.503$) on cumulative emissions for CH_4 and CO_2 during any of the time periods: day 0 to 14, day 14 to 28, day 0 to 28, day 28 to 42, and day 0 to 42 (Table 5). The steers fed RAC showed a 14.48% reduction for NH_3 for day 14 to 28 ($P = 0.046$) and 17.21% for day 0 to 28 ($P = 0.032$; Table 5) compared to CON. The steers fed RAC tended to reduce NH_3 emissions for day 0 to 14 with a 22.58% reduction ($P = 0.066$) and day 0 to 42 with an 11.07% reduction ($P = 0.070$; Table 5). Hydrogen sulfide in steers fed RAC vs. CON was reduced by 29.49% from day 0 to 14 ($P = 0.009$) and tended to be reduced over day 0 to 28 by 11.14% ($P = 0.086$; Table 5). No reductions for H_2S in the steers fed RAC vs. CON were found for day 14 to 28, day 28 to 42, or day 0 to 42.

Ractopamine had no effect on cumulative CH_4 and CO_2 emissions standardized for LW for any time period (Table 6). Ammonia emissions (NH_3) were reduced in RAC compared to CON when standardized for LW by 23.88% ($P = 0.018$) for day 0 to 14, 17.80% for day 0 to 28 ($P = 0.006$), and 12.5% ($P = 0.027$) for day 0 to 42 (Table 6). Hydrogen sulfide when standardized for LW was reduced in RAC compared to CON by 28.81% from day 0 to 14 ($P = 0.008$) and showed a tendency for a 11.32% reduction from day 0 to 28 ($P = 0.090$; Table 6). No differences ($P = 0.450$) were noted for steers fed RAC vs. CON for H_2S when standardized for LW from day 0 to 42 (Table 6).

Cumulative NH_3 standardized by HCW were reduced by 14.05% ($P = 0.013$; Table 7) for RAC compared to CON. All other cumulative gaseous emissions standardized by HCW were not different ($P \geq 0.350$) between treatments (Table 7).

Table 5. Effect of feeding ractopamine to steers for 42 d on cumulative gas emissions per head

	Treatment[1]			
	CON	RAC	SEM	P-value
NH_3, g/steer				
Day 0 to 14	869.36	673.04	50.50	0.066
Day 14 to 28	1,708.35	1,460.93	58.65	0.046
Day 0 to 28	2,577.70	2,133.98	89.42	0.032
Day 28 to 42	2,262.11	2,170.24	85.82	0.440
Day 0 to 42	4,839.82	4,304.22	164.32	0.070
CH_4, g/steer				
Day 0 to 14	4,476.68	4,277.25	290.65	0.645
Day 14 to 28	5,023.71	4,930.17	363.40	0.862
Day 0 to 28	9,500.39	9,207.42	644.38	0.759
Day 28 to 42	5,007.24	5,154.68	368.09	0.787
Day 0 to 42	14,507.63	14,362.10	983.24	0.920
CO_2, kg/steer				
Day 0 to 14	235.14	230.63	11.93	0.798
Day 14 to 28	292.96	287.04	16.01	0.803
Day 0 to 28	528.10	517.67	27.83	0.800
Day 28 to 42	299.72	300.33	18.56	0.981
Day 0 to 42	827.82	818.00	45.69	0.884
H_2S, g/steer				
Day 0 to 14	0.78	0.55	0.07	0.009
Day 14 to 28	2.81	2.64	0.22	0.440
Day 0 to 28	3.59	3.19	0.25	0.086
Day 28 to 42	5.74	5.57	0.48	0.803
Day 0 to 42	9.33	8.76	0.70	0.517

[1]Treatments: CON = finishing ration containing no RAC (Control); RAC = finishing ration containing 27.3 g/907 kg RAC DM basis (Ractopamine).

Table 6. Effect of Ractopamine on cumulative gas emissions per unit of LW in beef cattle

	Treatment[1]			
	CON	RAC	SEM	P-value
NH_3, g/kg LW				
Day 0 to 14	1.48	1.12	0.07	0.018
Day 0 to 28	4.21	3.46	0.13	0.006
Day 0 to 42	7.76	6.79	0.24	0.027
CH_4, g/kg LW				
Day 0 to 14	7.56	7.12	0.49	0.533
Day 0 to 28	15.55	14.91	1.06	0.686
Day 0 to 42	23.31	22.60	1.57	0.759
CO_2, kg/kg LW				
Day 0 to 14	0.40	0.37	0.02	0.626
Day 0 to 28	0.86	0.84	0.04	0.687
Day 0 to 42	1.32	1.28	0.07	0.676
H_2S, g/kg LW				
Day 0 to 14	0.00130	0.00093	0.0002	0.008
Day 0 to 28	0.00584	0.00518	0.0004	0.090
Day 0 to 42	0.01493	0.01383	0.0011	0.447

[1]Treatments: CON = finishing ration containing no RAC (Control); RAC = finishing ration containing 27.3 g/907 kg RAC DM basis (Ractopamine).

Growth performance and carcass characteristics

Initial BW were not different ($P = 0.770$) between treatments for CON and RAC-fed steers. Steers fed RAC vs. CON weighed 6.19 kg more on day 14 ($P = 0.022$) and tended to weigh 9.76 kg more on

Table 7. Effect of Ractopamine on cumulative gas emissions per unit of HCW in beef cattle

	Treatment[1]			
	CON	RAC	SEM	P-value
NH_3, g/kg HCW	13.19	11.33	0.37	0.013
CH_4, g/kg HCW	39.62	37.71	4.37	0.626
CO_2, kg/kg HCW	2.25	2.14	0.11	0.503
H_2S, g/kg HCW	0.025	0.023	0.002	0.348

[1]Treatments: CON = finishing ration containing no RAC (Control); RAC = finishing ration containing 27.3 g/907 kg RAC DM basis (Ractopamine).

Table 8. Effect of Ractopamine on the growth performance and feed intake (DM basis) of feedlot beef cattle

	Treatment[1]			
Item	CON	RAC	SEM	P-value
BW, kg				
Day 0	566.20	565.78	10.43	0.767
Day 14	592.55	598.74	10.26	0.022
Day 28	612.55	615.87	9.82	0.302
Day 42	624.31	634.07	10.17	0.107
ADG, kg/d				
Day 0 to 14	1.88	2.35	0.10	0.017
Day 14 to 28	1.44	1.22	0.15	0.255
Day 28 to 42	0.84	1.31	0.12	0.034
Day 0 to 42	1.39	1.63	0.08	0.066
DMI, kg/d				
Day 0 to 14	10.99	10.59	0.17	0.142
Day 14 to 28	10.94	10.44	0.13	0.031
Day 28 to 42	10.59	10.10	0.19	0.052
Day 0 to 42	10.84	10.38	0.15	0.069
G:F, DM basis				
Day 0 to 14	0.1711	0.2222	0.008	0.004
Day 14 to 28	0.1316	0.1116	0.014	0.167
Day 28 to 42	0.0792	0.1244	0.012	0.033
Day 0 to 42	0.1283	0.1539	0.007	0.012

[1]Treatments: CON = finishing ration containing no RAC (Control); RAC = finishing ration containing 27.3 g/907 kg RAC DM basis (Ractopamine).

day 42 ($P = 0.107$, Table 8). The steers fed RAC vs. CON showed a 0.47 kg/d greater ADG from day 0 to 14 ($P = 0.017$) and a 0.47 kg/d greater ADG from day 28 to 42 ($P = 0.034$; Table 8). From day 0 to 42 the steers fed RAC vs. CON tended ($P = 0.066$) to have a 0.24 kg/d greater ADG (Table 8). The steers fed RAC vs. CON consumed 4.52% less on a DM basis from day 14 to 28 ($P = 0.031$) and tended to consume 4.67% and 4.27% less from day 28 to 42 ($P = 0.052$) and day 0 to 42 ($P = 0.069$), respectively (Table 8). The steers fed RAC vs. CON increased G:F on a DM basis from day 0 to 14 with a 29.87% increase ($P = 0.004$), day 28 to 42 with a 57.07% increase ($P = 0.033$), and day 0 to 42 with a 19.95% increase ($P = 0.012$; Table 8).

Kidney, pelvic, and heart fat %, lean maturity, and overall maturity did not differ ($P \geq 0.170$) between RAC and CON. The steers fed RAC vs. CON had an average of 12.52 kg greater HCW ($P = 0.006$; Table 9). DP was 1.93 percentage units greater for RAC compared to CON ($P = 0.004$; Table 9). USDA YG was reduced for the steers fed RAC vs. CON ($P = 0.035$; Table 9). Fat thickness and marbling scores tended to be less for the steers fed RAC vs. CON with an 8.93% ($P = 0.057$) and 6.43% ($P = 0.060$) reduction,

Table 9. Effect of Ractopamine on quantitative carcass data of feedlot beef cattle

	Treatment[1]			
Item	CON	RAC	SEM	P-value
HCW, kg	367.21	379.73	6.58	0.006
DP, %	54.82	55.88	0.16	0.004
USDA YG	2.86	2.64	0.05	0.035
Fat Thickness, cm	1.42	0.51	0.01	0.057
REA, cm^2	89.74	93.87	1.68	0.100
KPH, %	1.70	1.67	0.07	0.717
Skeletal Maturity[2]	151.65	146.42	1.02	0.011
Lean Maturity[2]	142.94	145.96	1.37	0.170
Overall Maturity[2]	147.29	146.19	1.03	0.480
Marbling Score[3]	560.07	524.04	11.00	0.060

[1]Treatments: CON = finishing ration containing no RAC (Control); RAC = finishing ration containing 27.3 g/907 kg RAC DM basis (Ractopamine).
[2]The following are skeletal, lean, and overall maturity description with corresponding numerical scores: E = 500–599; D = 400–499; C = 300–399; B = 200–299; A = 100–199.
[3]The following are marbling descriptions with corresponding numerical scores: Very abundant (VAB) = 1000–1099; Abundant (AB) = 900–999; Moderately abundant (MAB) = 800–899; Slightly abundant (SLAB) = 700–799; Moderate (MD) = 600–699; Modest (MT) = 500–599; Small (SM) = 400–499; Slight (SL) = 300–399; Traces (TR) = 200–299; Practically devoid (PD) = 100–199.

respectively (Table 9). REA tended to be to be greater by 4.60% for the steers fed RAC vs. CON ($P = 0.100$, Table 9).

There were no differences ($P \geq 0.150$) for steers fed RAC vs. CON in the percent of carcasses that graded USDA prime, choice, and select (Table 10). There was a tendency ($P = 0.080$) for an increased percentage of carcasses that graded USDA Yield Grade 1 for steers fed RAC compared to CON (Table 11). There was no difference ($P \geq 0.120$) between treatments for the percent of carcasses that graded USDA Yield Grade 2, 3, or 4 (Table 11).

Discussion

The current study indicated no effect of RAC on the number of adverse health events. The only mortality in the current study was in the CON group. The literature supports results from this study as there are very few studies indicating that supplementation of RAC affects the number of adverse health events. The Freedom of Information Act for the FDA approval of the pioneer RAC showed no detrimental animal health effects at the labeled dosage of the product (Elanco, 2003). The generic RAC (Actogain 45, Zoetis) used in this study has the same label as the pioneer product. It is unlikely any of the abnormal health events observed during the treatment period were due to supplementation with RAC.

Ammonia is an air and water pollutant contributing to eutrophication, reduced visibility, soil acidity, and $PM_{2.5}$ formation (USEPA, 2004). There are three approaches suggested to decrease NH_3 loss from cattle: diet manipulation, manure per slurry treatment, and capturing and treating emitted gases (Hristov et al., 2011). Because open dirt floored corrals are the most common type of facility for feedlots, capturing and treating emitted gases is not a feasible solution. The addition of a βAA such as RAC to the cattle ration may be considered to suppress NH_3 losses. There are very few studies on βAA use to mitigate NH_3 emissions. The Freedom of Information Act for the

Table 10. Effect of Ractopamine on USDA QG of feedlot beef cattle

	Treatment[1]		
Item	CON	RAC	P-value
USDA QG, %			
Prime	3.64	0.00	0.496
Choice	94.54	86.80	0.273
Select	1.82	13.20	0.148

[1]Treatments: CON = finishing ration containing no RAC (Control); RAC = finishing ration containing 27.3 g/907 kg RAC DM basis (Ractopamine).

Table 11. Effect of RAC on frequency distribution of USDA YG in feedlot beef cattle

	Treatment[1]		
Item	Control	RAC	P-value
USDA YG, %			
1	1.8	11.3	0.082
2	58.2	64.2	0.527
3	36.4	22.6	0.124
4	3.6	1.9	0.588

[1]Treatments: CON = finishing ration containing no RAC (Control); RAC = finishing ration containing 27.3 g/907 kg RAC DM basis (Ractopamine).

FDA approval of lubabegron (Experior) as a method to reduce NH_3 emissions from feedlot cattle. Lubabegron, similar to RAC, was shown to reduce cumulative NH_3 emissions by 11.86% at a dose of 5 g/907 kg and showed a 15.94% reduction in g NH_3/kg HCW from the lubabegron-treated group compared to the control group over a 91-d test period (Elanco, 2018). The results reported for feeding lubabegron for reduction in cumulative NH_3 emissions and for NH_3 emissions standardized by HCW were very similar to the reductions observed in the present study, however, lubabegron can be fed for a longer period of time than RAC labeled usage of 42 d (Elanco, 2018). Stackhouse-Lawson et al. (2013) reported a reduction in NH_3 emissions per kg of HCW for cattle supplemented with a combination of monensin, tylosin phosphate, growth implants, and zilpaterol hydrochloride compared to control cattle, which were not supplemented with any additive or technology. A life cycle assessment conducted by Stackhouse-Lawson et al. (2012) modeled a 6% NH_3 reduction from Angus cattle treated with a β_2-adrenergic agonist (β_2AA; Zilpaterol, Merck Animal Health, Madison, NJ) compared to Angus cattle with no supplementation.

Beta agonists have been well studied for their ability to improve finishing cattle performance, particularly in regard to weight gain and increased carcass yields. Animals that reach slaughter weight at a faster rate spend less time on feed and produce less emissions overall. A meta-analysis looking at performance characteristics of feedlot cattle supplemented with βAA found that on average cattle supplemented with RAC had about 8 kg increase in BW, a 0.19 kg/d increase in ADG, and no substantial difference in DMI compared to cattle not supplemented with RAC (Lean et al., 2014). Strydom et al. (2009) saw a 23.81% increase in ADG with Bonsmara steers fed RAC at a rate of 30 ppm in feed for the last 30 d of finishing compared to the control group; however, ADG, DMI, and dressing % were similar. Abney et al. (2007) reported a 14.8% greater increase in ADG when RAC was fed for 35 d compared to 28 d, but similar ADG were seen from cattle supplemented for 35 d and 42 d. The results seen by Abney et al. (2007) suggest that a plateau is eventually reached as perhaps there is desensitization of the βAA receptors (Johnson et al., 2014).

Spiehs et al. (2015) found that steers fed a protein rich diet and supplemented with zilpaterol hydrochloride at a rate of 84 mg^{-1} $animal^{-1}$ d^{-1} had approximately a 24% lower flux of H_2S emission from feces compared to CON animals. The suggested mechanism for βAA ability to reduce H_2S emissions is the improved efficiency of the animal in feed to gain conversions (Spiehs et al., 2015). This corresponds to what was seen in the current study, during the first 14 d there was a greater ADG and lower H_2S in the RAC supplemented group vs. CON. However, as the study progressed, the ADG response was decreased compared to the first 14 d and the significant reduction in H_2S was no longer seen. When inhaled at high levels H_2S can cause oxygen deprivation and can be fatal to both animals and humans (Gerasimon et al., 2007). Fatalities associated with H_2S inhalation most commonly occur in confined animal housing situations where manure is stored in an anaerobic system such as a manure storage pit (Mitloehner and Calvo, 2008). While H_2S is of a smaller concern for human and animal health in outdoor dirt feedlots, the potential for RAC to reduce H_2S could be more beneficial in areas such as the Midwest in the United States where cattle are fed on slats indoors with manure storage pits below the animals in the barn.

Overall RAC shows great potential for mitigating NH_3 and improving steer performance and efficiency. Given that livestock are one of the largest contributors of NH_3 emissions in the United States, comprising 50% of the total NH_3 from terrestrial systems (NRC, 2003), RAC could play a major role in reducing the environmental footprint of beef cattle in feedlots.

Conclusion

As the agricultural sector continues to strive to create a more sustainable food system, it is important to consider how to maximize production, reduce the environmental impact of production, and provide safe and affordable nutrition to consumers. Ractopamine did not have an effect on CH_4, N_2O, or CO_2; however, it shows potential for reducing H_2S emissions which should be studied further. Ractopamine is a valuable tool to reducing NH_3 emissions and for improving beef cattle performance.

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Conflict of interest statement

The other authors declare no conflict of interest.

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