EVALUATION OF AN ENTERIC METHANE EMISSIONS MEASUREMENT SYSTEM FOR CATTLE

By

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EVALUATION OF AN ENTERIC METHANE

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Abstract: Growing concern about climate change and sustainability has increased societal pressures toward livestock production to quantify and reduce its environmental impact. Through the process of enteric fermentation, ruminant animals produce methane (CH_4), a potent greenhouse gas (GHG). To improve emission inventories and evaluate mitigation techniques, several methods of measuring emissions from ruminants have been developed. The present study evaluated a ventilated head box system capable of measuring CH_4 and carbon dioxide (CO_2) emissions, and oxygen (O_2) consumption from cattle. Six Holstein heifers were used to measure CH_4 and CO_2 emissions and O_2 consumption from two *ad libitum* intake measurement periods and one measurement period with intake restricted to 2% of body weight on a dry matter basis. As a measure of comfort in the head box system, all cattle were assessed for lying time, and respiration rates and THI were evaluated for thermal comfort. Methane and CO_2 emissions during the restricted intake period were significantly lower ($P < 0.0001$) than the *ad libitum* periods. Daily CH_4 emission rates per animal were reported as 235.0 ± 6.19 L/day, 228.3 ± 6.18 L/day, and 193.2 ± 8.88 L/day for the first and second *ad libitum* and feed restriction periods, respectively. Carbon dioxide emission rates were reported as 3627.5 ± 90.72 L/day, 3632.4 ± 90.47, and 3184.0 ± 104.79 L/day for the first and second *ad libitum* and feed restriction periods, respectively. Oxygen consumption rates were reported as 3390.59 ± 99.77 L/day, 3453.90 ± 99.57 L/day, and 3001.81 ± 111.36 L/day for the first and second *ad libitum* and feed restriction periods, respectively. Lying time was similar to behaviors reported in previous literature and averaged 779.17 ± 31.19 min/day, 768.79 ± 31.19 min/day, and 842.78 ± 31.19 min/day for the first and second *ad libitum* and restriction periods, respectively. There was no difference ($P > 0.05$) in THI and respiration rate across all measurement periods, and THI and respiration rate were positively correlated ($R^2 = 0.381$; $P < 0.0001$). The head box system provides an accurate method of measuring emissions from cattle and can provide information about daily variations and peaks in emissions.

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CHAPTER I

ENTERIC METHANE EMISSIONS MEASUREMENT AND PREDICTION FROM CATTLE

INTRODUCTION

As public concern about climate change and sustainability continue to grow, societal pressures on livestock production to quantify and reduce its environmental impact are rising. Livestock production, specifically ruminant production, is only one of many contributors to the production of greenhouse gas (GHG) emissions. Through the process of enteric fermentation, ruminant animals produce methane (CH_4), a potent GHG. Methane has a global warming potential 28 times that of carbon dioxide (CO_2) over a 100-year period (IPCC, 2013), and enteric fermentation is the source of 26% of the anthropogenic CH_4 emissions in the United States (US EPA, 2015). Therefore, CH_4 accounts for a large portion of the total GHG emissions from livestock production. Several methods of measuring emissions from ruminants have been developed to quantify and estimate emissions in order to improve emission inventories and evaluate mitigation techniques. These methods vary in complexity, accuracy, expense, and application (e.g. suited for numerous or single animals, grazing or housed livestock). The following review presents a brief history of enteric CH_4 emissions measurement from ruminants. In addition, methods of measuring and estimating emissions focused on beef cattle production, along with advantages and disadvantages of each technique, will be discussed.

ENTERIC METHANE EMISSIONS RESEARCH AND PRODUCTION

In the late 18th century, Antoine Laurent Lavoisier is credited to have been one of the first scientists to study the relationship between metabolism and the production of heat in animals. Lavoisier and Pierre-Simon LaPlace determined that the major part of heat in an animal is produced from the combustion of oxygen with organic substances in the body (Kleiber, 1961). From the research of Lavoisier, LaPlace and others, the generalization that life is a combustion process was developed. The concept relating metabolism to combustion led to research objectives that established relationships between gas exchange and heat production, determined the basis for the evaluation of feeds that could be related to energy requirements and expenditures, and established the causes of energy expenditures (Johnson et al., 2003). Adam Crawford constructed a combustion calorimeter by the suggestions of Joseph Priestley to measure animal heat, and around the same time, Lavoisier and LaPlace also constructed an animal calorimeter like that of Crawford's (Kleiber, 1961). However, Lavoisier and LaPlace measured the latent heat of melting ice rather than measuring sensible heat like Crawford (Kleiber, 1961). The development of the bomb calorimeter enabled the measurement of the heat of combustion of feeds and fuels and the determination of the gross energy content of organic compounds, feeds, feces and urine (Kleiber, 1961; Johnson et al., 2003). Wilbur O. Atwater and Edward B. Rosa built one of the most well known animal respiration calorimeters in 1899, and demonstrated that heat production and work of humans is derived from the chemical energy of catabolized material (Kleiber, 1961). Henry P. Armsby's respiration calorimeter for steers was an offspring of the Atwater calorimeter, but for larger animals (Kleiber, 1961). Through energy balance experiments using respiration calorimetry, relationships between gas exchange and heat production were established.

Research pertaining to the energy metabolism of animals led to an interest in ingested gross energy lost as eructated CH_4. Metabolizable energy is determined by subtracting the heat of combustion of the fecal matter, urine and CH_4 produced after feed consumption from the heat

combustion of a feedstuff (Blaxter and Clapperton, 1965). Determining metabolizable energy depends on measurements of energy losses. Energy loss in feces and urine can be determined with animals housed in metabolism cages by conducting total collections of urine and feces; however, measurement of the gaseous losses, such as CH_4 production, is more difficult, costly, and requires complex equipment (Blaxter and Clapperton, 1965; Moe and Tyrrell, 1979). Determining energy loss in the form of CH_4 emissions requires either adequate direct measurements of CH_4 production or means of estimating CH_4 production through the use of prediction equations (Blaxter and Clapperton, 1965; Moe and Tyrrell, 1979). Cattle lose approximately 2 to 12% of gross energy intake as eructated CH_4; hence, research has focused on reducing enteric methane emissions to improve production efficiency (Johnson and Johnson, 1995). More recently, concerns about the amount of ingested gross energy cattle lose as CH_4 emissions have coupled with concerns for the climatic impact CH_4 imposes on the environment. As a consequence, there have been efforts to improve enteric CH_4 emissions measurement and estimation methods.

Enteric Methane Production

Most CH_4 produced in the beef industry originates from the digestive tract of the animal or anaerobic manure storage systems. Methane is produced predominantly in the rumen and to a small extent in the large intestine of ruminants. The variation in CH_4 losses (approximately 2 to 12% of GE intake) is primarily caused by the amount of dietary carbohydrate fermented and the available hydrogen (H_2) supply through the ratio of volatile fatty acids (VFA) produced (Johnson and Johnson, 1995). In the rumen, the microbial community ferments carbohydrates, proteins and lipids to produce VFAs and other longer-chain fatty acids, as well as H_2 and CO_2 (Ellis et al., 2008; Janssen, 2010). The end products of microbial fermentation, including VFAs, H_2 and CO_2, are either used by the animal after absorption through the rumen wall, or used as substrates for other microbes in the rumen (Ellis et al., 2008). Carbon dioxide is additionally expelled via the

mouth of the animal. The three main VFAs produced in the rumen are acetate, butyrate, and propionate. Propionate is utilized in the production of glucose via gluconeogensis, and it is crucial to efficient growth and milk production (Place et al., 2011a). Acetate and butyrate are essential to the production of fat or oxidation for energy as described by Place et al. (2011a). Undigested feed components, VFAs not absorbed through the rumen wall, and microbial cells enter the remainder of the digestive tract (Janssen and Kirs, 2008; Janssen, 2010). The fermentation products of H_2 and CO_2 represent the major substrates used by methanogenic archaea, or methanogens. Methanogens use H_2 in an anaerobic respiration process to produce energy, which produces CH_4 as an end product (Ellis et al., 2008; Janssen, 2010). The methanogens' utilization of H_2 to reduce CO_2 to CH_4 keeps the H_2 concentration in the rumen low (Figure 1) (Place et al., 2011a). Hydrogen is also utilized in the production of propionate (Ellis et al., 2008). Propionate competes for the use of H_2 in the rumen and an increase in propionate production relative to acetate and butyrate production can lead to a decrease in overall CH_4 production (Ellis et al., 2008). Although methanogens are only a small part of the rumen microbial biomass, they are important for the removal of H_2, which can lead to a more favorable formation of VFA and eliminate the inhibitory effect of H_2 on the rate of fermentation (Janssen and Kirs, 2008). Methane is released from the rumen through the mouth of the animal by the process of eructation or belching. Additionally, CH_4 produced in the large intestine through fermentation in the hindgut is either released from the animal through absorption in the bloodstream and then expired, or is released from the rectum (Murray et al., 1975; Ellis et al., 2008). In experiments by Murray et al. (1975), very small amounts of CH_4 were collected at the rectum of sheep and were mostly voided when the animal defecated. Approximately 2 – 3% of total CH_4 emissions are expelled from the hindgut via the rectum (Murray et al., 1975; Muñoz et al., 2012).

METHODS OF METHANE EMISSIONS MEASUREMENT AND PREDICTION

Numerous methods have been developed to estimate and measure the emission of CH_4 from cattle. Such methods differ in their complexity and accuracy. The selection of a measurement technique may be influenced by the complexity of the system, cost, housing required, mobility, or labor intensity. The following discussion will outline the numerous methods and provide a comparison of advantages and disadvantages (also summarized in Table 1).

Indirect Calorimetry

Most of the available information relating to CH_4 emissions from cattle has been collected through the use of respiration calorimetry techniques, such as ventilated head boxes, facemasks and whole animal chambers (Johnson and Johnson, 1995). Indirect calorimetry and direct calorimetry techniques have been developed to study animal bioenergetics as outlined in the previous section. Indirect calorimetry measurements are used to calculate heat production from O_2 consumption, CO_2 production, CH_4 production, and urinary nitrogen using the Brouwer equation; direct calorimetry measurements determine the heat loss from an animal directly (Kleiber, 1961; Johnson et al., 2003). Most indirect calorimetry systems are open-circuit, while a few are closed-circuit systems. Open-circuit indirect-respiration techniques (i.e. head boxes or whole-animal chambers) circulate outside air around the animal's head, mouth, and nose and collect the expired air (McLean and Tobin, 1987). Outside air enters the collection apparatus (i.e. head box or whole-animal chamber) through inlet openings, and expired air is drawn through the system by negative pressure created with a pump (Kleiber, 1961; McLean and Tobin, 1987). A diagram of an open-circuit respiration chamber is shown in Figure 2. Total airflow through the system is measured and the difference in concentration of CH_4 gas between inspired and expired air is calculated to determine CH_4 emissions (Johnson and Johnson, 1995). It has been reported that the air entering the apparatus must be the same composition as the air flowing though the inlet channels, therefore if air enters through leaks or another inlet opening, gaseous

measurements will not be affected as long as suction is maintained and no chamber air exits or leaks from the chamber (Kleiber, 1961). Therefore, open-circuit systems have an advantage over closed-circuit systems because leaks into the whole-animal chamber or head box do not negatively impact CH_4 emissions. However, leaks occurring along the outlet air lines leading from the head box or whole-animal chamber to the gas analyzing equipment interfere with accurate CH_4 emission measures.

Ventilated head box-type systems have the capability to accurately measure gases eructated from the rumen in real-time measurements and calculate emission rates (Kelly et al., 1993; Suzuki et al., 2007; Odongo et al., 2008; Place et al., 2011b). The ventilated head box method uses a canopy to cover the head, or the upper part of the body. Ambient air enters the head box either through one or more specific inlets or gaps between the head box and the animal. In most systems, the danger of losing expired air is negligible because of the slight negative pressure environment in the head box created by drawing air through the head box by a vacuum pump (McLean and Tobin, 1987). Head box systems designed with clear polycarbonate siding or windows ensure animals have visual contact with animals housed in neighboring boxes or stalls to warrant consistent dry matter intake when the animals are housed in the head box compared to their natural environment and to reduce stress (Suzuki et al., 2007; Odongo et al., 2008; Place et al., 2011b). Head box systems have a cost advantage over whole-animal respiration chambers and an accuracy advantage over the sulfur hexafluoride (SF_6) technique when measuring CH_4 emissions (Johnson and Johnson, 1995). However, hindgut CH_4 expelled from the rectum cannot be measured with the head box system.

Emissions from the cattle in the head box system are calculated from the measured outlet gas concentration of each head box, background or ambient air gas concentrations, and the air flow rate through the head boxes (Place et al., 2011b). The volume emission rate (ER) at standard temperature and pressure is calculated from the net concentration and sampling flow rate of the

head box system (Place et al., 2011b; eq 1). The net concentration of the gas is the difference between the outlet gas concentration and the ambient gas concentration (Place et al., 2011b).

$$ER = \ Net\ Concentration\ (ppm) x\ Sampling\ flow\ rate\ \left(\frac{L}{minute}\right) \quad [1]$$

Whole-animal chambers are regarded as the gold standard method of measurement for CH_4 emissions because the instruments are reliable, stability of the instruments can be measured, and the environment of the chamber can be controlled (McLean and Tobin, 1987; Johnson and Johnson, 1995). Whole animal chambers are useful in experiments measuring CH_4 emissions from ruminal and hindgut fermentation. However, chambers can create a non-natural environment and affect animal behavior, such as dry matter intake. Dry matter intake is a main driver of enteric CH_4 emissions, and a decrease in dry mater intake would affect total CH_4 emissions, as well as derived estimates like loss of gross energy via enteric CH_4 emissions (Storm et al., 2012). Additionally, whole-animal chambers can be costly to construct, are not portable, and require large buildings to house them. Chambers may have a disadvantage in applying results to grazing animal emissions estimates due to the restriction in animal movement, which may influence feeding behavior. Feeding behaviors of animals confined to chambers may differ than that of animals able to move about in a pasture. Whole-animal chamber and head box systems can provide information on the variability of emissions within a day allowing researchers to determine times of highest and lowest emissions on an individual animal basis (i.e. emissions are typically highest after feeding; Grainger et al., 2007).

To validate and test the accuracy and precision of data collected from head box and chamber systems, recovery tests can be performed. Recovery tests confirm that there are no leaks from the system, and emissions from the animal are being accurately measured (Suzuki et al., 2007). Recovery tests are performed by injecting a known amount of gas into the system at a constant flow rate, and accounting for background concentrations before and after the injection of

the gas (Suzuki et al., 2007; Place et al., 2011b). The recovery percent is calculated from the total injected and total recovered amounts of gas. Before any measurements are made with a system, recommendations from Suzuki et al. (2007) state that the system should achieve 95 – 105% recovery values.

A measurement system using a mouthpiece or facemask, rather than a head-box, will work similar to the ventilated-head box system. The animal inhales and exhales directly into the mouthpiece of the collection apparatus (McLean and Tobin, 1987). There are many disadvantages of using a facemask compared to head boxes and whole-animal chambers. Whole animal chambers have the capability to collect foregut and hindgut emissions from the animal, while the facemask, like a head box system, is limited to eructated emissions. Furthermore, facemasks will limit the movement of the animal, which may impact their ability to eat and drink.

In closed-circuit systems, the animal is placed in a closed chamber with CO_2 and moisture absorbers, and the quantity of O_2 needed to replace that used by the animal is measured as it is admitted to the system (McLean and Tobin, 1987). Oxygen is replaced continuously as it is used in the chamber; CH_4 accumulates in the chamber, and the production rate is calculated from samples taken at the beginning and end of the collection period (Blaxter and Clapperton, 1965; McLean and Tobin, 1987). The O_2 consumed and CO_2 produced are calculated from these analyses (McLean and Tobin, 1987). Methane production is determined as the difference between the final and initial volumes of CH_4 sampled in the chamber (Blaxter and Clapperton, 1965). Heat production can be calculated by either substituting the measured gas quantities directly into the Brouwer equation or from the carbon-nitrogen balance by sampling and analyzing recorded feed intake and excreta for carbon and nitrogen content (McLean and Tobin, 1987). Using the carbon-nitrogen balance method of calculation eliminates the need for O_2 analysis and was useful before the invention of paramagnetic O_2 analyzers (McLean and Tobin, 1987). Closed-circuit systems are suited for metabolic measurements on small animals and are more difficult for use with large

animals, such as cattle. The challenge of using closed-circuit systems for large animals, such as cattle, can be attributed to the volume of gases consumed and produced by large animals.

SF_6 Tracer Technique

The SF_6 tracer gas technique allows animals to move about in their natural environment, while directly measuring enteric CH_4 emissions. A permeation tube with a known release rate of the SF_6 tracer gas is placed into the rumen and a halter with a capillary tube connected to a sampling canister is placed on the animal's head (Johnson and Johnson, 1995). The animals must be trained to wear the collection canister and halter, and the halter and canister must fit properly on the animal in order to avoid movement of the halter that could potential obstruct the drinking and feeding behaviors of the animal. The concentration of gases released from the mouth and nose of the animal can be quantified using SF_6 as a marker, which is released at a known rate (Boadi et al., 2002). With the tracer method, SF_6 is utilized to account for the dilution of gases as they exit the animal's mouth and mix with ambient air (Johnson et al., 1994). The dilution rates for SF_6 and CH_4 are assumed to be identical due to the assumption that the SF_6 emission from eructation simulates the CH_4 emission from eructation (Johnson et al., 1994). The rate at which CH_4 is emitted can be calculated from the measured CH_4 and SF_6 concentrations and the known release rate of SF_6 from the permeation tube in the rumen (eq 2). Methane emissions (Q_{CH_4}; g/d) are calculated using the SF_6 and CH_4 mixing ratio sampled by the canisters (C_{SF_6} and C_{CH_4}, respectively; mmol/mol) and the inlet air streams ($C^b_{SF_6}$ and $C^b_{CH_4}$, respectively), and the predetermined SF_6 release rate (Q_{SF_6}; g/d) from the permeation tube where the ratio of molecular weights (MW) is used to account for the difference in density between the gases (Grainger et al., 2007; Johnson et al., 1994; McGinn et al., 2006).

$$Q_{CH_4} = \frac{C_{CH_4} - C^b_{CH_4}}{C_{SF_6} - C^b_{SF_6}} Q_{SF_6} \frac{MW_{CH_4}}{MW_{SF_6}} \quad [2]$$

Multiple animals can be sampled simultaneously without restraint creating an advantage to using the SF_6 tracer technique compared to other methods that require an animal to be restrained (i.e. chambers, head boxes and facemasks). However, animals have to be trained to wear the halter and collection canister, and hindgut methane is not measured.

When comparing the use of chambers to the SF_6 technique to measure CH_4 emissions, total CH_4 emissions were similar (McGinn et al., 2006; Grainger et al., 2007). However, the SF_6 tracer gas technique underestimated CH_4 emissions over a range of diets by an average of 4-8% relative to the chamber technique (McGinn et al., 2006; Grainger et al., 2007). Some of the underestimation of the SF_6 technique may be attributed to the gas recovery within the collection canisters, as well as post-ruminal CH_4 loss that is captured within whole-animal chambers but not the SF_6 technique (McGinn et al., 2006). Additionally, the SF_6 permeation rate is positively associated with the CH_4 emission estimates leading to variability in CH_4 emission estimates by the tracer technique (Pinares-Patiño and Clark, 2008). The SF_6 tracer gas technique is best suited for use in grazing animals as it has been shown that agreement between the chamber and SF_6 techniques is highest when cattle are fed high forage diets at a restricted level of intake (McGinn et al., 2006). An additional consideration when using the SF_6 tracer gas technique, as well as face masks and head boxes, is the use of rumen cannulated animals. Beauchemin et al. (2012) showed leakage of SF_6 and CH_4 from the rumen cannula of animals with the proportion of SF_6 and CH_4 recovered at the head of the animal differing ($P < 0.001$) by cannula type. The SF_6 tracer gas technique, as well as face masks and head boxes, is not recommended to be used with cannulated cattle as gas may exit the rumen due to leakage from the cannula (Beauchemin et al., 2012).

Additional Techniques

A system called GreenFeed (C-lock Inc., Rapid City, South Dakota, USA) combines an automated feeding system with the capability of measuring CH_4 and CO_2 emissions (Storm et al.,

2012). As animals enter the feeder, they are recognized, a small amount of feed is dispensed to keep the animal at the feeder for a number of minutes, and CH_4 and CO_2 concentrations are measured. To quantify airflow and the emitted CH_4 and CO_2 during the visit to the GreenFeed, air is continuously pumped through the automatic feeding system (Storm et al., 2012). The GreenFeed system automatically performs recovery experiments by releasing small amounts of a known tracer gas inside the feeder's head cabin to determine how much of the expired air from the animal is captured by the system during visits to the feeder (Storm et al., 2012). The GreenFeed system is portable and applicable to use in a grazing or tie-stall situations. As a disadvantage, the system only measures CH_4 emissions when an animal is directly eating from the feeder.

In a study done by Hammond et al. (2015), the GreenFeed system was compared to respiration chambers and the SF_6 technique. Hammond et al. (2015) reported a non-significant association ($P > 0.50$) between the GreenFeed and chamber system. Correlation coefficients between the GreenFeed system and chambers were 0.1043 and 0.058 when used to measure CH_4 production and yield of individual heifers, respectively; however, the average overall CH_4 emissions were similar for both systems (Hammond et al., 2015). The authors also found less variability with chamber measurements of CH_4 emissions (g/day) compared to the GreenFeed data, but state this may be due in part to measurements of the GreenFeed system that were fewer in number and taken less frequently than the chamber measurements. The correlation coefficient between the GreenFeed system and the SF_6 technique to measure CH_4 production from individual heifers was 0.602, and there was a significant ($P < 0.01$) association between the two techniques (Hammond et al., 2015). Average overall CH_4 emissions were lower for the GreenFeed measurements than those of the SF_6 technique, and Hammond et al. (2015) determined the differences might be due to the duration of the CH_4 measurements obtained for each animal.

Large variations in CH_4 emission rates throughout a day will make accurate estimations of daily CH_4 emissions difficult when snapshot sampling, such as that used by the GreenFeed system, is used (Jonker et al., 2014). A study by Jonker et al. (2014) observed a more pronounced circadian variation of CH_4 emissions for animals fed a restricted amount of feed and at fewer feeding frequencies than animals offered feed for *ad libitum* intake. The CH_4 emission profile after each feeding for animals on restricted feed intake reached a peak within 40 minutes of the feeding and gradually declined until the next feeding (Jonker et al., 2014). To estimate daily CH_4 emissions using the GreenFeed system, snapshots of CH_4 emissions spread over the whole 24 hours of a day should be taken to reflect daily emissions due to the variation in emissions throughout a day (Jonker et al., 2014). If animals are not inclined to visit the GreenFeed during a meal, throughout the day and night, or visit immediately following a meal, accurate estimation of daily CH_4 emission rates may be difficult. The reduced circadian variation in CH_4 emissions seen with *ad libitum* feeding by Jonker et al. (2014) suggests the use of the GreenFeed system may provide the most accurate daily CH_4 emission estimates when animals have *ad libitum* feed available.

The micrometeorological mass difference technique measures differences in the concentration of gases in the atmosphere and relates these fluxes to animal emissions (Harper et al., 1999). Methane concentrations are calculated from measurements of wind speeds and atmospheric CH_4 concentrations on the upwind and downwind boundaries (Harper et al., 1999). The flux of CH_4 from the cattle is calculated by subtracting the upwind flux from the downwind flux. The different micrometeorological methods of measuring CH_4 emissions are impacted by instabilities such as non-steady state wind or movement of point-emission sources (Storm et al., 2012). The micrometeorological technique is a nonintrusive way to measure emissions from cattle while they remain in an environment in which they are accustomed. This technique can be used for grazing cattle or feedlot cattle. The micrometeorological methods are useful in

measuring whole system emissions, but further development and documentation of reliability is necessary (Storm et al., 2012).

In vitro methods for measuring CH_4 production from rumen fermentation have been developed to avoid expensive whole animal *in vivo* measurements of enteric CH_4 emissions. This technique can be done in a lab setting and does not require animals to be used. *In vivo* animal experiments done to measure CH_4 emissions can be costly, time consuming and require large specialized facilities and resources (Navarro-Villa et al., 2011). *In vitro* cumulative gas production techniques were developed to simulate and predict rumen fermentation of feed and feedstuffs (Rymer et al., 2005; Navarro-Villa et al., 2011; Pellikaan et al., 2011). Gas production is measured as an indication of fermentation from incubation of a feedstuff with buffered rumen fluid (Rymer et al., 2005). The incubated feedstuff is degraded and either fermented to produce gas and fermentation acids, or incorporated into microbial biomass (Rymer et al., 2005). Gas production techniques combined with measures of degradation provide a measure of the proportion of the feed that is fermented as opposed to that which is partitioned to microbial growth (Rymer et al., 2005). *In vitro* techniques do not account for long-term adaptation of ruminal microorganisms to the tested feedstuffs (Storm et al., 2012). Studies comparing the *in vitro* gas production technique to other methods of CH_4 measurement, such as chamber systems and the SF_6 technique, report varied results of agreement and non-agreement between the methods (Storm et al., 2012). Bhatta et al. (2006) compared the *in vitro* gas production technique to the SF_6 tracer method and found the correlation coefficient of CH_4 production between the two methods to be 0.85. Bhatta et al. (2008) reported weak correlations between the *in vitro* gas production technique and respiration chambers. *In vitro* CH_4 measurements after 24 hours (R^2 = 0.37), and measurements after 48 hours (R^2 = 0.24) were correlated with respiration chamber measurements recorded over 3 consecutive days.

Prediction Models

In addition to measuring emissions directly from cattle, there are numerous prediction models that can be used to estimate CH_4 emissions as an alternative to live animal experiments. Table 2 provides examples of common prediction models for enteric CH_4 emissions in beef production. Since it is a gaseous loss, measuring CH_4 emissions requires expensive equipment; therefore, the metabolizable energy of many diets is estimated from calculated rather than measured CH_4 production (Moe and Tyrrell, 1979). The use of mathematical models to predict CH_4 emissions can help avoid extensive and costly experiments requiring cattle (Ellis et al., 2007). Developing prediction models for CH_4 production has focused on the relationship between CH_4 production and dry matter intake and dietary carbohydrates in the diet (Moe and Tyrrell, 1979).

Mathematical models can be classified as statistical models (e.g. relate nutrient intake to CH_4 production using correlations derived from empirical data) or dynamic mechanistic models (e.g. estimate CH_4 production using mathematical descriptions of rumen fermentation biochemistry; Kebreab et al., 2006). Numerous enteric CH_4 emissions prediction equations use the Intergovernmental Panel on Climate Change (IPCC) (2006) Tier 2 methodology, including Nguyen et al. (2010), Pelletier et al. (2010), and Casey and Holden (2006). Ellis et al. (2010) evaluated several enteric CH_4 emissions prediction models used in dairy whole farm system models, including the IPCC Tier 2 methodology with enteric CH_4 emission measurement data from live animal experiments. The authors found that overall the models had low prediction accuracy. Ellis et al. (2010) compared the equations based on mean square prediction errors (MSPE) and concordance correlation coefficient analysis (CCC), and found values ranging from 20.2 to 52.5 for MSPE and CCC values ranging from 0.000 to 0.493. All models evaluated had particular difficulty in predicting the wide range of enteric CH_4 emissions observed in live animal experiments, which are affected by diet type and level of intake (Ellis et al., 2010). Moraes et al. (2014) developed new enteric CH_4 prediction equations using a dataset from 2,574 indirect

calorimetry records from both beef and dairy cattle, and compared the newly developed models to IPCC Tier 2 and the Food and Agriculture Organization of the United Nations' (FAO) (2010) methodology. Across all classes of animals (heifers, lactating cows, dry cows, and steers), the authors found their new models that use gross energy intake, as the only user required input, consistently outperformed both the IPCC Tier 2 and FAO models (Moraes et al., 2014). As an example, the model fitted to the lactating cow data set with gross energy intake as the only covariate had substantially lower prediction error than the IPCC model (18.14 vs 30.50 %, respectively) (Moraes et al., 2014). As a caveat, the dataset did not include any emissions from grazing cattle nor did the diets fed to the steers contain the level of concentrates found in most feedlot cattle diets today (Moraes et al., 2014). The difficulty in collecting grazing cattle CH_4 emissions and the consequential relative dearth of enteric CH_4 emission data from grazing cattle in the literature is challenging for enteric CH_4 prediction equations. Without datasets to evaluate models, little can be known about the accuracy and precision of commonly used enteric CH_4 prediction equations for grazing cattle, which typically represents the largest share of CO_2-eq emissions in beef production.

CONCLUSION

Enteric CH_4 emissions account for a large portion of the total GHG emissions from ruminant livestock production, and the beef industry has faced increasing pressure to reduce its environmental impact. The discussed methods of measuring and estimating CH_4 emissions from ruminants (i.e. chambers, SF_6 tracer technique, and prediction models) have been developed to quantify and estimate emissions in order to improve emission inventories and evaluate mitigation techniques. Improvement of emission inventories and mathematical models predicting GHG emissions is important in reducing the uncertainty associated with the contribution of ruminants to the total global, as well as US, CH_4 emissions. The developed methods vary in complexity, accuracy, expense, and application (e.g. suited for numerous or single animals, grazing or housed

livestock). Indirect calorimetry techniques, such as whole-animal chambers and head box systems, are considered accurate and reliable. However, whole animal chambers can be expensive to construct, require extensive animal training and accurate extrapolation of results to grazing animal emissions is questionable. Using a ventilated head box system to measure CH_4 emissions from cattle provides a more cost effective method than whole-animal chambers and an accurate method of measurement. Further research should determine the accuracy and the effects of chambers on dry matter intake and emissions in relation to grazing versus housed animals. A study at Oklahoma State University, Stillwater, Okla., was developed to validate and further research the use of the ventilated head box systems. Cattle were used to assess the impact of the ventilated head box system on feed intake and animal behavior, and to determine the variation of CH_4 emissions within and between animals fed the same diet on an *ad libitum* and restricted basis. The results of the present study will help determine the impact of feed restriction on CH_4 production and the minimum amount of time required for animals to be housed in the head box system in order to achieve an accurate 24-hour emissions rate calculation for use in future studies.

Table 1. A comparison of the advantages and disadvantages of measurement and prediction techniques of methane (CH_4) emissions.

Technique	Advantages	Disadvantages
Whole-animal chambers	- Ability to measure CH_4 emissions from ruminal and hindgut fermentation	- Expensive to construct and maintain - Restriction of animal movement
Head box systems	- Lower cost of construction than whole animal chamber system - Ability to measure CH_4 emissions from ruminal fermentation	- Restriction of animal movement - Inability to measure hindgut CH_4 - May not account for emissions that leak from rumen cannulas if used with cannulated animals
SF_6 tracer technique	- Eliminates need to restrain animal; animal can move freely and graze - Multiple animals can be sampled simultaneously without restraint	- Animal must be trained to wear halter and collection canister - Inability to measure hindgut CH_4 - May not account for emissions that leak from rumen cannulas if used with cannulated animals
Face masks/Mouth piece	- Measures expired emissions from the animal	- Restriction of animal movement - Restricted drinking and feeding behaviors - Inability to measure hindgut CH_4 - May not account for emissions that leak from rumen cannulas if used with cannulated animals
GreenFeed (C-Lock, Inc.)	- Can be used in grazing or tie-stall situations - One GreenFeed unit can be used for numerous animals (~ 20 animals) - Animals are identified and emissions are measured for each animal when using the GreenFeed	- Does not capture continuous measurement like a chamber or head box system - Animals must be inclined to visit the GreedFeed multiple times a day, throughout the day - May not account for emissions that leak from rumen cannulas if used with cannulated animals
Micrometeorological mass difference	- Can be used in grazing or feedlot situations - Animals do not need to be trained	- Further research and documentation of reliability is needed - Difficult to have replicated experimental units

In vitro	- Does not require animals; can be less costly than chamber and head box systems	- May not be able to account for variations found in animal experiments
Prediction models	- Does not require animals; can be less costly than animal experiments	- May not be able to account for variations found in animal experiments

Table 2. Enteric methane (CH_4) prediction equations commonly used to estimate CH_4 emissions from beef production systems

Model	Reference(s)	Enteric methane (CH_4) emissions prediction equation
IPCC Tier 2	Casey and Holden (2006); Nguyen et al. (2010); Pelletier et al. (2010); Beauchemin et al. (2010);	CH_4/head/yr (kg)= (GE[1] x Y_m[2] x 365 days/yr) / (55.65[3] MJ/kg CH_4)
Shibata et al. (1993)	Ogino et al. (2004)	CH_4 (L/d) = -17.766 + 42.793 x (kg DMI[4]/d) – 0.849 x (kg DMI/d)^[2]
Mills et al. (2003)	Stackhouse-Lawson et al. (2012)	CH_4/head/day (kg) = [E_{max}[5] - E_{max}(-c[6] x M_{EI}[7])] x F_{kgCH4}[8]
Blaxter and Clapperton (1965)	Peters et al. (2010), grazing emissions	CH_4/head/day (kg) = 1.3 + 0.112DMD_{ijkl}[9] + L_{ijkl}[10] (2.37 - 0.050DMD_{ijkl})
Moe and Tyrrell (1979)	Capper (2011); Peters et al. (2010), feedlot emissions	CH_4/head/day (MJ) = 3.406 + 0.510 NFC[11] + 1.736 HC[12] + 2.648 C[13]

[1] GE = gross energy intake, MJ/head/day
[2] Y_m = methane conversion factor, percent of gross energy in feed converted to methane
[3] 55.65 (MJ/kg CH_4) is the percentage of gross dietary energy lost as methane
[4] Dry matter intake
[5] E_{max} = maximum possible emission, 45.98 MJ CH_4/head/day
[6] c = -0.0011 · [Starch/ADF] + 0.0045, Where: Starch = starch content of diet, ADF = acid detergent fiber content of diet
[7] M_{EI} = metabolizable energy intake, MJ/head/day
[8] F_{kgCH4} = conversion of MJ to kg of CH_4, 0.018 kg CH_4/MJ
[9] DMD_{ijkl} = digestibility of feed (expressed as a %)
[10] L_{ijkl} = feed intake relative to that needed for maintenance
[11] NFC = nonfiber carbohydrate (kg/d)
[12] HC = hemicellulose (kg/d)
[13] C = cellulose (kg/d)

Figure 1. Methanogens in the rumen keep the concentration of hydrogen gas (H_2) low by using H_2 to reduce carbon dioxide (CO_2) to methane (CH_4) through the process of methanogenesis.

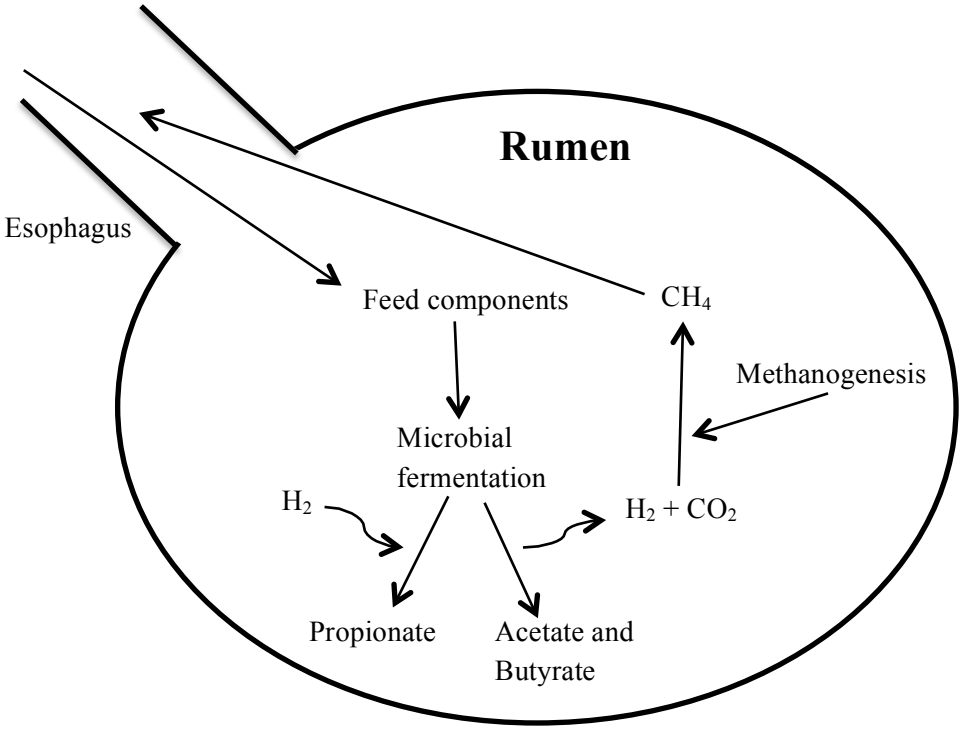
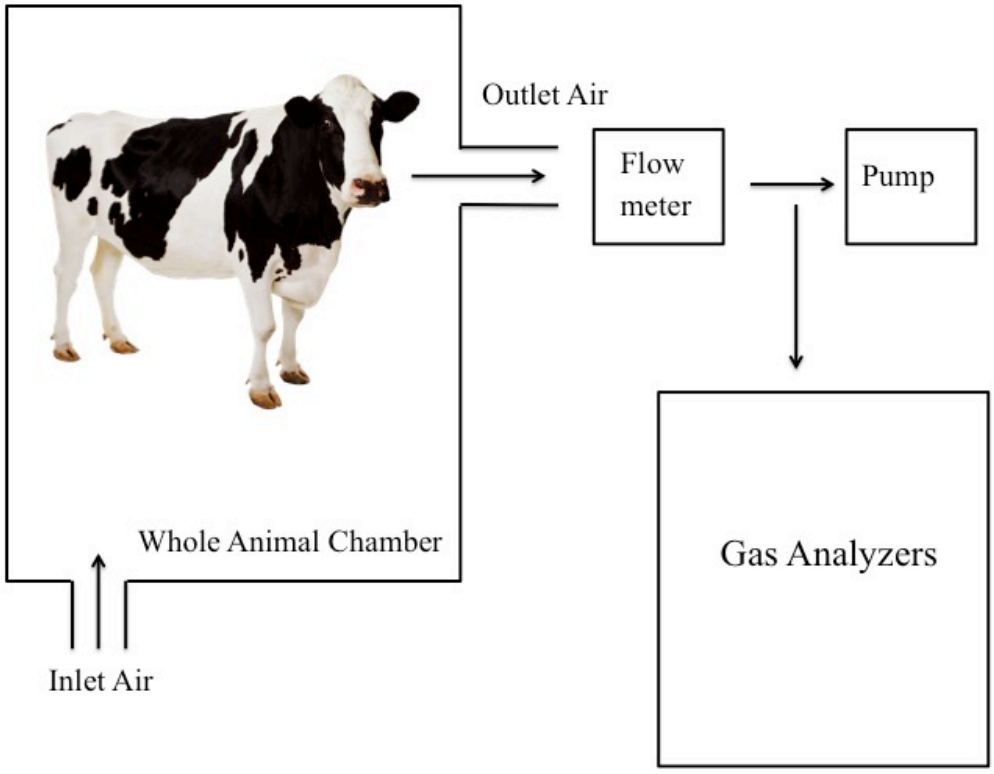


Figure 2. In an open-circuit respiration chamber, outside air enters the chamber through inlet openings, and expired air is drawn through the system by negative pressure created with a pump. The expired air is drawn through the gas analyzers for analysis.



CHAPTER II

EVALUATION OF AN ENTERIC METHANE EMISSIONS MEASUREMENT SYSTEM

INTRODUCTION

As public concern regarding climate change and sustainability continue to grow, rising societal pressures are forcing the livestock production industries to quantify and reduce their environmental impact. Livestock production, specifically ruminant production, is one of many agricultural and non-agricultural contributors to the emission of GHG. Ruminant animals produce CH_4, a potent GHG, through the process of enteric fermentation. Over a 100-year period, CH_4 has a global warming potential 28 times greater than CO_2 (IPCC, 2013), and enteric fermentation is the source of 26% of the anthropogenic CH_4 emissions in the United States (US EPA, 2015). Methane, therefore, accounts for a large portion of the total GHG emissions from livestock production in the United States. Several methods of measuring and estimating CH_4 emissions from ruminants (i.e. chambers, SF_6 tracer technique, and prediction models) have been developed to quantify and estimate emissions in order to improve emission inventories and evaluate mitigation techniques. These methods vary in complexity, accuracy, expense, and application (e.g. suited for numerous or single animals, grazing or penned livestock). Using a ventilated head box system to measure CH_4 emissions from cattle provides an accurate and cost effective method of measuring emissions from the mouth of the animal. In a ventilated head box system, the animal's head is placed in an airtight box and a canopy or neck sleeve is used to cover the head or

upper part of the body. Outside air is circulated around the animals' head, mouth, and nose, and the expired air is collected. Ambient air enters the head box through inlet openings, and expired air is drawn through the system by negative pressure created with a pump. Total airflow through the system is measured, and emissions are determined by calculating the difference in the concentration of gases between ambient air and expired air. A head box system has the capability of measuring gases eructated from the rumen in real-time measurements to calculate emission rates.

The objectives of the following study were to evaluate a ventilated head box system and to compare emissions across measurement periods of *ad libitum* and restricted feed intake. Additional objectives of the study were to determine the effect of the head boxes on measures of behavior (i.e. standing and lying behavior, dry matter intake (DMI), and respiration rate) and emissions. The results will provide information on the minimum amount of time required for animals to be housed in the head box system in order to calculate a 24-hour emissions rate for use in future studies.

MATERIALS AND METHODS

Study Overview

The study was conducted in accordance with the approved Oklahoma State University (OSU) Animal Care and Use Committee protocol (protocol number AG-13-16) at the OSU Nutrition Physiology Research Center in Stillwater, OK. Six Holstein heifers between 16 and 18 months of age (initial live weight between 364 and 430 kg) were obtained from the OSU Dairy to be used for the duration of the 72-day study. The heifers were weighed and paired based on weight. The heifers were used to assess the impact of the ventilated head box system on feed intake and behavior, and to determine the variation of CH_4 emissions within and between animals fed the same diet on an *ad libitum* and restricted basis. All 6 heifers were used in each period of

the study. In **period 1** (21 days), the heifers were fed in neighboring individual pens that allowed for visual and social contact between animals, as well as free-movement, and were provided *ad libitum* feed for 1 week. The following week, each animal was introduced to the head box and attached metabolism stall for three 2-hour training periods. During the third week of period 1, each animal spent three 6-hour training periods in the metabolism stall and head box. In **period 2** (3 days), each animal was housed in the metabolism stall and head box for the first 3-day gas measurement period and remained on *ad libitum* feed intake. After the first gas measurement phase, animals were returned to their individual pens and remained on *ad libitum* feed intake for **period 3** (21 days). In **period 4** (3 days), each animal was housed in the metabolism stall and head box for the second 3-day gas measurement period and remained on *ad libitum* feed intake. In **period 5** (17 days), animals were returned to their individual pens and remained on *ad libitum* feed intake. In **period 6** (4 days) the animals remained in their individual pens, and feed was restricted to 2% of their body weight on a dry matter basis. In **period 7** (3 days), each animal was housed in the metabolism stall and head box for a third and final 3-day gas measurement period and remained on restricted feed intake. Periods 1 – 7 are presented in Figure 3.

Head Box System Design and Operation

The system consisted of two ventilated head boxes, and a complete cattle respirometry system, which was housed in an instrumentation cabinet. A diagram depicting an overview of the ventilated head box system can be seen in Figure 4. The following sections will describe the dimensions and materials used to construct the head boxes, as well as the respirometry system used to calculate the emission rates from cattle.

Head Box Design

Two ventilated head boxes, similar in design to those in Place et al. (2011b), were constructed at the Oklahoma State University Biological and Agricultural Engineering Shop. The

head boxes were attached to metabolism stalls (96.5 cm wide) inside the OSU Nutrition Physiology Research Center barn and were placed side-by-side. The dimensions of the box measured 170.2 cm x 105.4 cm x 76.2 cm (H x W x D). Clear polycarbonate sheeting was used to provide full field of vision for the cattle on the back, front and two sides of the head box. The top and bottom of the box were made from stainless steel sheeting, and the frame was made from stainless steel angle iron. The bottom of the head box was designed similar to a pallet to facilitate easy movement of the box. The bottom measured 12.1 cm x 105.4 cm x 76.2 cm (H x W x D) making the total height of the box 182.3 cm. A float-type metal water bowl was attached 68.6 cm above the base along with an air inlet opening 7.6 cm in diameter on one side of the box. An extrusion, 2.5 cm in diameter, on the top of the box served as the air outlet. A door was placed on the front of the box to enable the feeding of animals or moving of animals in and out of the head box. To ensure an air tight seal during gas sampling, foam tape was placed along the edge of the door frame, and the door was closed with three locking catches on the top and bottom and four locking catches on the side. On the back of the box was an oval neck opening measuring 101.6 cm tall and 66.04 cm wide where the heifer's head entered the box. A Cordura fabric neck sleeve was attached to the head opening via a rolled steel attachment. The neck sleeve had a zipper on the top and bottom of the sleeve as well as a rope sewed into the end for easy placement and tightening over the animal's head and neck. A chain inside the head box was attached to each side of the base, and another chain with a clip on the end was attached to the middle. When an animal entered the head box, a chain around their neck was attached to the clip on the end of the chain inside the box. The chain was long enough to allow the animal to stand up, lie down, eat, and drink. Figures 5 and 6 are photographs of the head box when empty and in use during a gas collection phase, respectively.

Gas Sampling System and Operation

A complete cattle respirometry system with the capability of measuring CH_4 and CO_2 emissions and O_2 consumption was purchased from Sable Systems Inc. (Las Vegas, NV). The key components of the respirometry system included two integrated vacuum pumps and mass flow controllers called FlowKits, a SS-4 Sub-Sampler Pump, an infrared MA-10 Methane Analyzer and infrared CA-10 Carbon Dioxide Analyzer, a fuel cell FC-10 Oxygen Analyzer, a RH-300 Water Vapor Analyzer, an analog signal multiplexer, a flow multiplexer, a UI-2 Data Interface, and the Expedata data acquisition and analysis software. The respirometry system was housed in an instrumentation cabinet that was kept at approximately 26.7 °C by a small window A/C unit that was placed on the side of the cabinet (Figure 7). The air inside the head box was drawn through the outlet at the top through a filter and into the FlowKit that supplied an air-flow rate of 725 L/min. Subsamples were taken from each FlowKit and an ambient air sample was drawn from a pump located adjacent to the head boxes and partitioned into individual channels of the flow multiplexer. Air was pulled from a common port on the flow multiplexer by the SS-4 Sub-Sampler Pump, and the SS-4 pushed the air through the RH-300, the CA-10, MA-10, and FC-10 analyzers. The analog outputs of each FlowKit, the SS-4, RH-300, CA-10, MA-10, and FC-10 are sent to the UI-2 data interface, which is connected to the computer. Using the Expedata software, 65 minute sampling files were set to sample each of the head boxes and the ambient air every 5 minutes. Each file started with a 5 minute sample of ambient air, switched to sample head box 1 for 5 minutes, and then switched to sample head box 2 for 5 minutes. Sampling rotated from each of the air sampling locations (i.e. ambient air, head box 1, head box 2). The files were set to record continuously for the entire 72-hour sampling period and saved in a Dropbox file when completed to allow access to the data from numerous locations. A screen shot of the computer monitor was taken and updated in a Dropbox file every 5 minutes to allow the equipment to be remotely monitored.

Before the system was used in a gas collection phase, the recovery rate was calculated for each head box. Recovery rate calculations were determined by injecting a 99.99% CO_2 standard gas into each head box at 2.5 and 5 L/min for about 30 minutes. Sampling before and after the CO_2 injection was used to determine background concentrations of CO_2. Figure 8 is an example of a recovery rate test injection of CO_2. The recovery rates calculated for the boxes ranged from 96.70 to 111.68%. The calculated recovery rates are similar to those previously reported by others using head box systems. Suzuki et al. (2007) reported recovery rates from 95.7 to 101.8% for a four head box system, and Place et al. (2011b.) reported recovery rates from 97.6 to 99.3% for a two head box system. Table 3 summarizes the recovery rate tests for head boxes 1 and 2. Since the mean recovery rate for head box 2 was around 108%, a correction factor of 1.08 used for emission rate calculations during the experiment. To verify that there was not a leak in the system, recovery tests were conducted with the FlowKits switched for the two head boxes, and the same recoveries were calculated. The higher recovery percent for head box 2 was recognized as a mass flow rate recording issue with the FlowKit. Standard deviations for recovery percent for head box 1 and 2 (0.0196 and 0.0168, respectively) are similar, and to confirm the recovery percent had not changed over the course of the experiment, additional recovery tests were performed after completion of the experiment. The recovery tests conducted after the experiment resulted in the same recovery rate of approximately 108%. Emissions rates were calculated with the following equation:

$$ER = (sum(n)Q)\times(Cout - Cin))/n$$

Where ER is the gas emission rate from the head box (L/animal/minute), Cout is the average volume per minute concentration in the outlet air, Cin is the average volume per minute concentration in the ambient air, Q is the sampling flow rate (L/min), and n is the number of total effective measurements. The net concentration of the gas is the difference between the outlet gas concentration and the ambient gas concentration.

To calculate the L/hour emissions rate, the L/min value was multiplied by 60, and the L/hour value was multiplied by 24 to calculate the L/day emissions rate. Estimates were converted from volume per unit time to mass per unit time for comparison to other studies. The following equation is an example of converting CH_4 from volume per unit time to mass per unit time:

$$\frac{L}{unit\ time} \times \frac{1\ mol}{22.4\ L} \times \frac{16.04\ g\ CH_4}{1\ mol} = \frac{gCH_4}{unit\ time}$$

All spans and zero calibrations were done within 24 hours before the start of the gas measurement period. The RH-300 water vapor analyzer was zeroed by pulling pure nitrogen gas from a cylinder and allowing the nitrogen gas to flow for at least 30 minutes to verify that the display reached a low, stable value before setting the zero. The CA-10 and MA-10 analyzers were zeroed with pure nitrogen gas at the same time the RH-300 was zeroed. The span point was set for the CA-10 analyzer using 1% CO_2, balance nitrogen gas. The 1% CO_2 gas was allowed to flow through the CA-10 for at least 1 minute until the value on the display approached the value of the calibration gas. When the CA-10 reading stabilized, the span was set for 0 – 1% CO_2. The MA-10 analyzer span point was set using 1% CH_4, balance nitrogen gas. The 1% CH_4 gas was allowed to flow through the MA-10 for at least 1 minute until the value on the display approached the value of the calibration gas. When the MA-10 reading stabilized, the span was set for 0 – 1% CH_4. Setting the O_2 span for the FC-10 analyzer required dry, CO_2-free air. Two Drierite drying tubes filled with Drierite and one drying tube filled with Soda Lime were used. Air was pulled through a Drierite tube, the Soda Lime tube, then another Drierite tube before entering the SS-4, RH-300, CA-10, MA-10, and FC-10. After the CO_2 reading reached a low, stable value and the O_2 reading stabilized, the span point was set at 20.95% to span the FC-10 analyzer with ambient air.

Animal Handling during Training and Gas Measurement Periods

During the two-hour training periods (period 1), the animals were led into the stall and head box. The cattle were restrained by a head gate that restricted side-to-side, forward and backward movement but allowed the animal to stand and lie down. The heifer's head was placed in the head gate, and the head gate was tightened. The neck sleeve was placed and tightened around the animal's neck. The heifer's neck chain was attached to the floor chain in the head box, leaving the animal with enough chain length to access water, feed, and to lie down. The door of the head box remained open for the duration of the two-hour training period. Heifers were offered free choice feed and water and were monitored the entire time during the training period. The animals were returned to their individual pens after the training. For the six-hour training periods (period 1), the same protocol was followed as for the two-hour training sessions; however, the head box door was closed and latched for the duration of the six-hour training and was only opened if necessary.

Within 24 hours before the start of each gas measurement period when the sampling system was calibrated, the stalls were bedded with straw for animal comfort. At the beginning of each gas measurement period, the heifers were moved into the head boxes following the same protocol as the training periods. For all three measurement periods (periods 2, 4, and 7), the heifers were assigned the same head box each time. After the doors of each box were latched, the Expedata file was loaded, and the sampling system was started. A student remained with the animals at all times for the duration of the 72-hour sampling periods. Any time a head box door was opened, the time the door was opened and closed was recorded, and those data were not used in emission rate calculations.

Feed Intake Measures

The animals were offered a total mixed ration (TMR) for the duration of the study. Ingredients and composition of the TMR are listed in Table 4. The heifers were fed twice daily at

07:30 and 14:00 hr. During periods 1 – 5, the animals were fed *ad libitum* with targeted refusals of 5% on an as-fed basis. Refusals were collected and weighed before the morning feeding to calculate daily feed intakes. During the first and second gas measurement periods (2 and 4), the heifers were fed twice daily, and refusals were collected before the morning feeding. For periods 6 and 7, the feed intake of the heifers was restricted to 2% of their body weight on a dry matter basis. The animals were fed once daily at 07:30 hr at the start of feed restriction in period 6 and throughout the final measurement period 7. Refusals were collected before the morning feeding as usual. Feed intake is commonly restricted beginning 4 days before measurement periods and continuing throughout the measurement period (Boadi and Wittenberg, 2001; Van Zijderveld et al., 2011a, 2011b). Literature suggests feed restriction should begin before the start of the measurement period due to the incidence of gut fill, which can impact CH_4 production (Boadi and Wittenberg, 2001; Van Zijderveld et al., 2011a, 2011b). Throughout the study, water was available for *ad libitum* intake.

Dry matter was calculated weekly by drying feed samples in a drying oven for 48 hours at 60°C. During the gas collection periods, a sample of refusals was collected for each animal for dry matter analysis each day. For each new batch of TMR, a representative sample was frozen for future analysis. At the end of the study, a composite sample from all batches of frozen TMR was made and sent to DairyOne Forage Laboratory (Ithaca, NY) for laboratory analysis. The composite TMR was made from samples of equal weight from each batch of frozen TMR.

Behavior Measures

Respiration Rates

Respiration rates were measured as the number of flank movements per minute (Mitlöhner et al., 2001). Flank movements were counted for 30 seconds and multiplied by 2 to calculate the total per minute for each animal. Respiration rates were taken at both the morning

and afternoon feedings and were used as a measure of behavior. Before the beginning of each training period, respiration rates were taken while the animal was still in its individual pen before being moved to the head box for the training session. Heifers were moved back to individual pens after each training session and before respiration rates were collected for the afternoon feeding time. During the measurement periods (2, 4, 7), respiration rates were taken at the normal morning and afternoon feeding times while the heifers were in the head boxes for the collection.

Activity Data Loggers

Onset Pendant G data loggers (Onset Computer Corporation, Bourne, MA) were used to measure the frequency and duration of standing and lying behaviors as a measure of cattle comfort for the experiment. The Y and Z-axis of each data logger was set to record at 5-minute intervals following the recommendations of Ledgerwood et al. (2010). Loggers were wrapped in shelf liner and then with vet wrap to protect the logger, label the logger with a number, and provide some cushion between the logger and the heifers' legs. Loggers were placed with the x-axis horizontal to the ground on the outside of the right hind leg and in the middle of the leg (below the hock and above the metatarsophalangeal joint), as recommended by Ledgerwood et al. (2010). The animal's leg was first wrapped with a thick layer of cotton roll, followed by two to three layers of vet wrap. Gorilla glue was placed on the back of each logger wrapped in shelf liner and vet wrap. Then, the logger was placed on top of the animal's vet wrapped-leg and an additional two to three layers of vet wrap were placed over the logger to secure the device on the leg. Figure 9 shows an attached logger on a heifer. Loggers were attached at the beginning of the experiment and removed and re-attached at the middle of the study to check the data, placement of the loggers, and legs of each animal. Loggers were removed at the end of the study.

Temperature-Humidity Index

To calculate the temperature-humidity index (THI), temperature and relative humidity were recorded using Onset Pro V2 temp/RH loggers (Onset Computer Corporation, Bourne, MA). One temp/RH logger was placed adjacent to the animals individual feeding pens, and another temp/RH logger was placed adjacent to the head boxes inside the barn to account for any differences in temperature and relative humidity in the outside pen area or in the barn. The temp/RH loggers were set to record temperature and relative humidity at 10-minute intervals for the duration of the study. Temperature-humidity index was calculated using the following equation from Mader et al. (2006):

$$THI = 0.8 \times ambient\ temperature + [(\%\ relative\ humidity\ \div 100) \times (ambient\ temperature - 14.4)] + 46.4$$

Data collected by the Pendant G loggers and by the temp/RH loggers were downloaded using Onset HOBOware Software (Onset Computer Corporation) and exported to Microsoft Excel for further analysis.

Chute Temperament and Exit Score

While the animals were being weighed at the beginning and end of the study, each was evaluated and given a chute temperament score while they were in the chute and an exit score as they left the chute. Chute temperament scores were based on the scoring system used by Grandin et al. (1995). Once the animals were in the head catch, they were observed by a trained observer for 15 seconds without any human interaction. The chute score was based on the 15-second observation period. Exit scores were based on the scoring system used by Lanier and Grandin (2002) as the animals were leaving the chute. The same trained individual evaluated chute temperament and exit score for the duration of the study to minimize variation between observers.

Tables 5 and 6 display the score values and definitions used to determine the chute temperament and exit scores of the cattle.

Pen Temperament

Pen temperament was scored weekly based on the scoring system used in Hammond et al. (1996). Table 7 represents the scoring system used to score pen temperaments of the cattle. Since the cattle were housed in individual pens, the cattle were scored individually in their pens. To score the cattle, a trained observer entered the pen, and if the animal did not notice the observer immediately, the observer walked counter-clockwise along the perimeter of the pen until the heifer was aware of the observer. When the heifer was aware of the observer, the observer stopped and took one step toward the heifer due to the small size of the pen. Each heifer's response was evaluated to assess the temperament of the heifers in their pen. The same individual scored pen temperament for the duration of the study in order to minimize variation between observers.

Statistical Analysis

All data were analyzed using the Proc Glimmix procedure in SAS version 9.4 (SAS Institute Inc., Cary, NC). The CH_4, CO_2 and O_2 models included period, day within period, hour, DMI, hour by period interaction, hour by day within period interaction, DMI by period interaction, and DMI by day within period interaction as fixed effects, and heifer as a random effect. Feed intake data was modeled with DMI as the dependent variable, period, day, and day within period as fixed effects, and heifer as a random effect. The CH_4 g / kg of DMI was modeled with the ratio of CH_4 g/day to kg of DMI as the dependent variable, period, day within period, hour, hour by period interaction, hour by day within period interaction as fixed effects, and heifer as a random effect. Logger data was modeled with standing time, lying time, standing bouts, lying bouts, standing duration, and lying duration as dependent variables, period, day, and period

by day interaction as fixed effects, and heifer as a random effect. Respiration rate was modeled with average daily respiration rate as the dependent variable, daily THI mean, period, and location as fixed effects, and heifer as a random effect. Temperature-humidity index was modeled with average daily THI as the dependent variable, period, and location as fixed effects, and heifer as a random effect. The Proc Corr procedure of SAS version 9.4 (SAS Institute Inc., Cary, NC) was used to evaluate the correlation of THI and respiration rate. Residual versus predicted plots were examined to check the assumption of normality for all models. Least-squares means were compared across periods using a Tukey adjustment. Comparisons were considered statistically significant when $P < 0.05$. Results are presented as the least squares means estimates ± standard error. The period variable used for data analysis consisted of the 7 periods previously described. The location variable used for data analysis consisted of 2 locations: the outside location used to describe periods 1, 3, 5, and 6 when animals were housed in their individual pens, and the inside location used to describe periods 2, 4, and 7 when animals were housed inside the barn in the head boxes during gas measurement periods.

RESULTS

Emissions Measurements

There was no difference in CH_4 emissions between periods 2 and 4 ($P = 0.0759$) of *ad libitum* intake, which were higher than period 7 ($P < 0.0001$) of restricted intake. For CO_2 emissions, period 7 was lower ($P < 0.0001$) than periods 2 and 4, which were not different ($P = 0.9789$). Oxygen consumption during period 7 was lower than that of periods 2 and 4 ($P < 0.0001$), and period 2 was lower ($P = 0.0190$) than period 4. Table 8 presents the least squares means of CH_4, CO_2, O_2, DMI, and g CH_4 / kg of DMI for day within period. There were no differences ($P > 0.05$) between days for L/day estimates in periods 4 and 7 for CH_4, CO_2, and O_2 (Figures 10 - 12). The hour by period interactions for CH_4, CO_2 and O_2 are illustrated in Figures

13 - 15. For all emissions, the hour by period interaction of the model was significant ($P < 0.0001$). There was no difference ($P > 0.05$) across periods in CH_4 emissions for hours 3 through 7 and 10 through 12, CO_2 emissions for hours 2, 4 through 6, and O_2 consumption for hours 1, 2, 4 through 6 between feeding 1 and 2. The peaks of CH_4 and CO_2 emissions after feedings and significant interactions of hour are shown in Figures 13 and 14. Figures 16 - 18 display the significant interactions of period by hour for CH_4 (L/day) estimates. The CH_4 (L/day) estimates for hours 13 through 24 were different ($P < 0.05$) than the daily estimates of other hours. The variation in emission estimates seen throughout the 24-hour cycle of a day (Figures 16 - 18) highlights the need to measure emissions for a full 24 hours in order to estimate CH_4 emissions per day. Period and day within period for CH_4 g per kg DMI were significant ($P < 0.0001$). Periods 4 and 7 were not different for CH_4 (g/kg DMI) estimates ($P < 0.0949$). Period 2 was higher than period 4 ($P < 0.0040$) and was higher than period 7 ($P < 0.0001$).

Feed Intake Measures

There was no difference ($P = 0.3264$) in DMI between periods 2 and 4. Dry matter intakes for period 7 were lower ($P < 0.0001$) than period 2, which was expected as heifers were fed a restricted amount during periods 6 and 7. However, DMI for period 7 did not differ ($P = 0.0884$) from period 4. Period 1 was higher ($P < 0.0001$) than period 2, and period 3 was higher ($P < 0.0001$) than period 4. However, there was no difference ($P = 0.2449$) between period 1 and 4. Average DMI for periods 2 (9.2 ± 0.450 kg) and 4 (9.8 ± 0.450 kg) was lower than the average DMI of periods 1 (10.4 ± 0.407 kg), 3 (11.0 ± 0.407 kg), and 5 (11.9 ± 0.409 kg). There was no difference in DMI between periods 6 (8.4 ± 0.438 kg) and 7 (8.4 ± 0.450 kg). There was no difference ($P > 0.05$) across days 1, 2, and 3 within periods 2, 4, and 7.

Behavior Measures

Figures 19 - 24 illustrate the trends in standing and lying behaviors, as they changed over the three-day gas measurement periods when compared to the mean standing and lying behaviors of the animals when they were in their individual feeding pens. The standing and lying behaviors of the animals during individual feeding periods 1, 3, 5, and 6 were averaged and are represented as the period labeled "outside". For the standing and lying time variables, there was a difference between the outside period compared to day 1 of period 2 ($P = 0.0079$) and period 4 ($P = 0.0026$). The second and third day of periods 2, 4, and 7 did not differ from the outside period for mean standing and lying time. Overall, there was a numerical increase in the number of standing and lying bouts during periods 2, 4, and 7 compared to the outside period, as well as a numerical decrease in mean standing and lying bout duration during periods 2, 4, and 7. Compared to the outside period, there were a higher number of standing bouts per day for day 1 ($P < 0.0001$) and day 2 ($P = 0.03$) of period 2; day 1 ($P < 0.0001$) and day 2 ($P = 0.024$) of period 4; day 1 ($P < 0.0001$), day 2 ($P = 0.0006$), and day 3 ($P = 0.0152$) of period 7. Lying bout duration of the outside period was higher ($P < 0.05$) than all measurement period days, except for day 3 of period 2.

The least-squares means and differences of THI and respiration rate for each period are presented in Tables 9 and 10. Period 1 was different ($P < 0.0001$) than all other periods. There was no difference in THI between periods 2 and 4 ($P = 0.1302$), periods 2 and 7 ($P = 0.3335$), and periods 4 and 7 ($P = 0.9993$). There was no difference in respiration rate between periods 2 and 4 ($P = 0.6917$), periods 2 and 7 ($P = 0.8348$), and periods 4 and 7 ($P = 1$). A positive correlation ($R^2 = 0.381$; $P < 0.0001$) was found between THI and respiration rate. For all periods, THI remained at a normal level (≤ 74) according to the Livestock Weather Safety Index (LWSI) classifications for heat stress (Mader et al., 2006). There was no change in pen temperament scores throughout the study, as well as no change in chute temperament and exit scores from the

beginning to the end of the study (data not shown). The animals remained docile and non-aggressive throughout the study.

DISCUSSION

Emissions Measurements

In a study done by Jonker et al. (2014) using heifers that were 20 months old and 382 ± 24.3 kg body weight, the authors found the CH_4 emissions profile after each feeding for restricted feed intake periods followed an asymmetrical negatively skewed shape. Peak emissions were attained within 40 minutes of feedings and gradually declined until the next feeding (Jonker et al., 2014), which was similar to the peak and decline in emissions after each feeding seen in the present study (Figure 13). Jonker et al. (2014) concluded that low daily DMI with infrequent feeding (restricted feeding) can increase circadian variation in CH_4 emission rates within a day, but *ad libitum* feeding reduced the circadian variation in CH_4 emissions. For cows on *ad libitum* feed, variation between minimal and peak CH_4 emission rates were less than when feed was restricted (Jonker et al., 2014). Jonker et al. (2014) reported CH_4 yield was similar among all periods (restricted and *ad libitum* feed intake; $P = 0.63$), and averaged 24.3 ± 1.23 g/kg DM. The CH_4 yield reported in the current study averaged 18.07 ± 0.48, 17.45 ± 0.48, and 17.05 ± 0.48 g/kg DM for periods 2, 4, and 7, respectively. In a study by Jiao et al. (2013), average CH_4 emissions per kg of intake was 23.5 g/kg in 6-month-old Holstein cattle. The authors reported average CH_4 emissions per day of 96.4 g/day for steers and 90.5 g/day for heifers, and there was no difference ($P = 0.32$) between the heifers and steers. These daily emissions are lower than our reported CH_4 emissions per day, which ranged from 127.60 to 174.05 g/day. Beauchemin and McGinn (2005) measured the CH_4 emissions from Angus heifers fed backgrounding and finishing diets containing corn or barley, and observed higher emissions when cattle were fed a backgrounding diet than when fed a finishing diet. Emissions reported ranged from 62.1 g CH_4

/day for cattle fed a corn finishing diet to 170.6 g CH_4 /day for cattle fed a corn backgrounding diet (Beauchemin and McGinn, 2005). The backgrounding diets used by Beauchemin and McGinn (2005) consisted of 70% whole crop barely silage, 25% steam-rolled barely grain, and 5% supplement for the barley diet, and 70% corn silage, 18% dry-rolled corn grain, and 12% supplement for the corn diet on a dry matter basis. The finishing diet used by Beauchemin and McGinn (2005) consisted of 9% barley silage, 81.4% steam-rolled barley or dry-rolled corn, and 9.6% supplement on a dry matter basis. The emission rates observed in the present study are similar to the higher emissions observed in the backgrounding phase by Beauchemin and McGinn (2005). The backgrounding diets used by Beauchemin and McGinn (2005) contained slightly higher roughage percentages than the diet used in this current study. Methane emissions per kg DMI observed by Beauchemin and McGinn (2005) ranged from 9.2 g CH_4/kg DMI for corn finishing diets to 24.8 g CH_4/kg DMI for corn backgrounding diets. The CH_4 emissions per kg DMI seen in the present study are in the middle of the range observed by Beauchemin and McGinn (2005). Chiavegato et al. (2015) conducted a 2 x 2 Latin square experiment in which Holstein steers (542 – 589 kg body weight) received one dietary treatment for period 1 and the other treatment for period 2, and also reported lower CH_4 emissions than those reported from this study. The authors reported that steers fed a 10% crude protein diet averaged 84.6 and 77.7 g CH_4 /day for period 1 and 2, respectively, and averaged 9.4 and 7.7 g CH_4 /kg DMI for period 1 and 2, respectively. For steers fed a 13% crude protein diet, Chiavegato et al. (2015) reported CH_4 emissions averaging 99.3 g/day and 81.5 g/day and 10.2 g/kg DMI and 8.5 g/kg DMI for period 1 and 2, respectively. Freetly and Brown-Brandl (2013) measured CH_4 production over a 6-hour period and recommended the CH_4 emissions reported should not be used to represent daily CH_4 production. Steers produced 85.8 ± 4.6 g CH_4/day or 7.7 ± 0.6 g CH_4 / kg DMI (Freetly and Brown-Brandl, 2013). Heifers weighed 390 ± 11 kg, were under 1 year of age, and produced 95.8 ± 4.2 g CH_4/day or 13.0 ± 0.7 g CH_4 / kg DMI (Freetly and Brown-Brandl, 2013). Freetly and Brown-Brandl (2013) speculated their lower values in CH_4 emissions compared to other studies

may be due to sampling strategy, diet differences, and the heifers used were younger compared to other literature. As seen with our study, CH_4 emissions differed throughout the 24-hour cycle in the day, and measuring emissions for only 6 hours may not account for within day variation of CH_4 production. Hales et al. (2013) reported daily CH_4 emission rates between 69.8 and 101.9 L/animal and CO_2 emission rates between 2,692 and 2,968 L/animal for steers (322 kg body weight) fed steam-flaked, corn-based diets with wet distillers grains with solubles. Daily CH_4 emission rates per animal in this study were reported as 235.0 ± 6.19 L/day, 228.3 ± 6.18 L/day, and 193.2 ± 8.88 L/day for periods 2, 4, and 7, respectively, and CO_2 emission rates were reported as 3627.5 ± 90.72 L/day, 3632.4 ± 90.47, and 3184.0 ± 104.79 L/day for periods 2, 4, and 7, respectively. Some of the variation in emission rates seen from the literature compared to this study may be attributed to differences in dietary treatments, measurement strategies (i.e. measuring emissions for a full 24-hours compared to 6 hours), and animals used during the experiment (i.e. lactating dairy cows compared to growing steers or heifers).

Behavior Measures

Adequate opportunity to lie down and rest for dairy cattle is considered important for the well-being and comfort of cattle. Many factors can affect the lying behavior of cattle, such as stall surface and bedding, stall size, stocking density, stall location, pen layout, pen flooring, social ranking, production, and health status (Haley et al., 2000; Tucker et al., 2004; Jensen et al., 2005; Ito et al., 2009). It has been reported that dairy heifers are motivated to lie down for about 12 – 13 hours/day (Jensen et al., 2005). Ito et al. (2009) studied lying behaviors from 43 commercial dairy farms in British Columbia using free-stall barns, and reported that the average total lying time of cows was 11.0 ± 2.1 hours/day. The average lying time when heifers were in individual feeding pens, periods 1, 3, 5 and 6, was 870.43 ± 46.82 minutes/day or 14.5 hours/day. Therefore, the average lying times for periods 2, 4, and 7 were shorter than the average time when heifers were in individual feeding pens. Lying time averaged 779.17 ± 31.19 minutes/day (13 hours/day),

768.79 ± 31.19 minutes/day (12.8 hours/day), and 842.78 ± 31.19 minutes/day (14 hours/day) for periods 2, 4, and 7, respectively. Although lying times in periods 2, 4, and 7 were shorter than the average lying time of the heifers in their individual pens, the averages are not lower than the reported average lying time of dairy heifers. Tucker et al. (2004) assessed the impact of free-stall size on dairy cattle behavior, and reported that time spent lying down can provide information about how comfortable animals find a given stall design. According to the authors, animals spent more total time lying down and exhibited longer lying bouts when using wider stalls. Using 3 different stall widths, cows averaged at 13.0 hours/day in the widest stalls (126 cm wide), and 12.3 hours/day in the narrowest (106 cm wide) ($P = 0.02$) (Tucker et al., 2004). Haley et al. (2000) compared lying and standing behaviors of Holstein cows in tie-stalls (180 cm long x 130 cm wide) with concrete flooring and tethered by a neck chain to cows in larger pens (420 cm long x 390 cm wide) with a soft mattress flooring material. Cows spent significantly ($P = 0.0006$) more time lying down (14.73 ± 0.91 hours/day) in large pens; lying 40% more than in tie stalls (10.51 ± 1.03 hours/day) (Haley et al., 2000). In this study, the head boxes were attached to metabolism stalls that measured 96.5 cm wide. Although the metabolism stalls were not as wide as stalls previously studied, this study used dairy heifers, whereas Tucker et al. (2004) and Haley et al. (2000) observed lying behavior in dairy cows. The decrease in lying time while housed in the head boxes and metabolism stalls corresponds with previously reported literature of decreased lying time in narrower stalls; however, the overall average lying time for all periods did not vary greatly from the values reported on how long dairy cattle are motivated to lie down throughout a day in Haley et al. (2000) and Tucker et al. (2004). Haley et al. (2000) observed that cows spent significantly less ($P = 0.0006$) time standing in the large pens (8.53 ± 0.90 hours/day) compared to the tie stalls (12.80 ± 1.05 hours/day), which is expected since standing time is the inverse of lying time. For periods 1, 3, 5, and 6, standing times averaged 568.04 ± 46.85 minutes/day or 9.5 hours/day, and standing time averaged 660.83 ± 31.19 minutes/day (11 hours/day), 671.11 ± 31.19 minutes per day (11.2 hours), and 597.22 ± 31.19 minutes/day (10 hours/day) for periods 2,

4, and 7, respectively. Although standing time was numerically highest for period 4 and lowest when the heifers were in individual feeding pens, there was no significant difference ($P > 0.05$) in standing time across the periods.

Ito et al. (2009) reported the average number of lying bouts was 9 ± 3 bouts/day from cows in free-stall barns. The average number of lying bouts per day for the heifers during periods 1, 3, 5 and 6 was 13 ± 2.20 bouts/day. The average number of lying bouts was significantly different between periods ($P = 0.0043$), and number of bouts increased during periods 2, 4, and 7. Lying bouts averaged 18.8 ± 1.27 bouts/day, 19.5 ± 1.27 bouts/day, and 22.6 ± 1.27 bouts/day for periods 2, 4, and 7, respectively. Tucker et al. (2004) observed lying bouts that averaged 12.3 to 11.9 bouts/day ($P = 0.45$) for narrow to wide stalls, respectively. Haley et al. (2000) reported cows had significantly ($P = 0.0024$) more lying bouts per day (13.62 ± 1.45 bouts/day) in large pens compared to tie stalls (8.21 ± 1.16 bouts/day), which is the opposite of the results seen in this study. Haley et al. (2000) observed more ($P = 0.0014$) standing bouts in the large pens (15.29 ± 1.34 bouts/day) compared to the tie stalls (9.75 ± 1.07 bouts/day). In periods 1, 3, 5, and 6, standing bouts averaged 9.62 ± 1.93 bouts/day, and bouts averaged 18.22 ± 1.34 bouts/day, 17.72 ± 1.34 bouts/day, and 19.89 ± 1.34 bouts/day for periods 2, 4, and 7, respectively. There was no difference ($P > 0.05$) in standing bouts between periods 2, 4, and 7; however, the average of periods 1, 3, 5, and 6 was significantly lower ($P < 0.05$) than all other periods. In our study, a significant increase in the average number of lying and standing bouts per day was seen in periods 2, 4, and 7. Total lying time throughout the day was typical compared to other studies, but the number of bouts increased. The observed increase in lying bouts shows the heifers got up and down more throughout the day while housed in the head boxes compared to in their individual pens.

Average lying bout duration for cows in free-stall barns has been reported to be 88 ± 30 minutes/bout (Ito et al., 2009). The average lying bout duration for the heifers during periods 1, 3,

5 and 6 was 75.5 ± 5.81 minutes/bout. Average lying bout duration was significantly different ($P < 0.0001$) between periods, and periods 2, 4, and 7 had lower average bout durations. Lying bout duration averaged 46.4 ± 3.65 minutes/bout, 42.4 ± 3.65 minutes/bout, and 39.6 ± 3.65 minutes/bout for periods 2, 4, and 7, respectively. Duration of lying bouts averaged 1.1 to 1.2 hours/bout ($P = 0.04$) for narrow to wide stalls, respectively (Tucker et al., 2004). Lying bout duration was shorter ($P = 0.1530$) in large pens (68 ± 5.68 minutes/bout) compared to tie stalls (86.72 ± 13.20 minutes/bout) (Haley et al., 2000). The average lying bout duration seen in this study is much shorter in periods 2, 4, and 7 than average lying bout duration reported in previous studies. The decrease in lying bout duration relates to the increase in lying bouts in periods 2, 4, and 7. Haley et al. (2000) found standing bout duration was significantly shorter ($P = 0.0015$) in the large pens (36.14 ± 5.31 minutes/bout) than in the tie stalls (86.70 ± 13.23 minutes/ bout). For periods 1, 3, 5, and 6, standing bout duration averaged 63.09 ± 7.23 minutes/bout, and bout duration averaged 42.45 ± 4.92 minutes/bout, 39.61 ± 4.92 minutes/bout, and 30.53 ± 4.92 minutes/bout for periods 2, 4, and 7, respectively. There was no difference ($P > 0.05$) across periods 2, 4, and 7, but the average of periods 1, 3, 5, and 6 was significantly higher ($P < 0.05$) than all other periods. The increase in standing bout duration also relates to the increase in standing bouts seen in periods 2, 4, and 7.

Mitlöhner et al. (2001) reported respiration rates for feedlot cattle with treatments of shade and/or water misting during summer months in Texas. For cattle receiving shade and misting, respiration rates averaged 30.0 ± 3.28 flank movements/minute, and for cattle receiving shade only, rates averaged 33.0 ± 3.28 flank movements/minute (Mitlöhner et al., 2001). For cattle receiving no shade and no misting, rates averaged 46.67 ± 3.28 flank movements/minute (Mitlöhner et al., 2001). Respiration rates for our study were lowest in period 1 at 28 ± 0.57 flank movements/minute, and highest in period 4 at 36 ± 1.08 flank movements/minute. For this study, heifers were shaded while in their individual feeding pens, and the chamber measurements were

conducted inside of a barn. For all periods, THI remained at a normal level (≤ 74), and our study was conducted during the months of February through May. The THI for periods 2, 4, and 7 was higher than THI for periods 1, 3, 5, and 6 when animals were in their individual feeding pens, which correlates with the higher respiration rates for periods 2, 4, and 7 (Tables 9 and 10; Figure 25). The respiration rates seen in our study are similar to those seen by Mitlöhner et al. (2001) for cattle provided shade conditions. Valtorta et al. (1997) also reported that respiration rates of cows provided shade compared to non-shaded animals differed significantly ($P = 0.01$) and averaged 60.7 ± 10.57 flank movements/minute and 78.9 ± 18.04 flank movements/minute for shade and non-shaded animals, respectively. The rates reported by Valtorta et al. (1997) are higher than all rates reported in this study, however, Valtorta et al. (1997) reported THI averaged 73.1 ± 3.2 throughout the study, which is higher than the average THI reported in this study (Table 9). Roman-Ponce et al. (1976) reported average respiration rates for shaded and non-shaded cattle in Florida during the summer months. As others have observed, respiration rates were lower ($P < 0.01$) for shaded cattle at 54 flank movements/minute compared to non-shaded cattle at 82 flank movements/minute (Roman-Ponce et al., 1976). The average THI of periods 1, 3, 5, and 6 (56.2 ± 1.154) was lower ($P < 0.0001$) than the average THI of periods 2, 4, and 7 (65.5 ± 1.626). The increase in respiration rates seen in this study from periods 1, 3, 5, and 6 compared to periods 2, 4, and 7 may be attributed to the increase in THI from the outside pens to the inside of the barn where the head boxes were housed rather than attributed to an increase in respiration rate due to stress of the animal.

CONCLUSION

Although ruminant livestock production is one of many contributors to the production of GHG emissions, societal pressures to quantify and reduce the environmental impact of ruminants are continuing to grow. In order to improve GHG emission inventories and to evaluate mitigation techniques, accurate and reliable methods of measuring emissions are necessary. A ventilated

head box system to measure CH_4 and CO_2 emissions, as well as O_2 consumption, from cattle provides an accurate and cost-effective method of measurement. Emission measurements from the head box system can provide information about diurnal variations in emissions, peaks in emissions after feedings, and can help determine the minimum amount of time required for animals to be housed in the head box system in order to calculate a 24-hour emissions rate. For the two gas measurement periods where heifers remained on *ad libitum* feed intake, there was no difference in CH_4 emissions, and the emissions for the gas measurement period of restricted intake was significantly lower. Variation in emission rates throughout the 24-hour cycle of a day was observed with peaks in emissions occurring after feeding and a slow decline in emission rates after and between feedings. The observed variation in emission rates emphasizes the need to measure emissions for a full 24-cycle in order to accurately estimate daily emission rates. The effect of the head boxes on the animals was evaluated based on a number of behavior measures. There was no difference in DMI between the two gas measurement periods when the animals remained on *ad libitum* feed intake. Standing and lying behaviors of the cattle while housed in the head boxes did not differ greatly from previously reported literature. Lying time and lying bout duration increased from day 1 to day 3 of the three-day gas measurement periods, which could be indicative of the heifers adjusting to being housed in the head box. The respiration rates of the cattle, as well as THI, increased during periods of gas measurement, however there was a positive correlation between THI and respiration rates. The head box system will be useful in examining the effects of mitigation strategies on emissions, variation in emissions caused by different feeds, and the pattern of emissions throughout the day.

Table 3. Recovery rate calculations for head box 1 and 2.

Head Box	CO_2 Injection (L/min)	Mass Flow Rate (L/min)	Calculated Injected CO_2 (L)	Actual Injected CO_2 (L)	Recovery (%)
1	5	724	186.40	187.17	99.59
1	5	724	208.85	211.33	98.82
1	2.5	724	77.84	76.21	102.15
1	2.5	724	78.84	77.04	102.33
1	2.5	724	78.59	77.38	101.57
1	5	724	247.42	251.92	98.22
1	5	724	235.14	243.17	96.70
1	5	724	251.40	256.08	98.17
1	5	724	182.26	182.58	99.82
Mean (± standard deviation) recovery rate head box 1					**99.71 (± 0.0196)**
2	5	723	219.77	204.08	107.69
2	5	723	219.94	205.67	106.94
2	5	723	219.58	203	108.17
2	2.5	723	85.57	78.29	109.30
2	2.5	723	76.64	68.63	111.68
2	5	724	143.27	133	107.72
2	5	724	136.46	128.33	106.33
2	5	724	139.83	130.33	107.29
Mean (± standard deviation) recovery rate head box 2					**108.14 (± 0.0168)**

Table 4. Ingredients and composition of the total mixed ration on a dry matter basis.

Item	% DM
Feed Ingredient	
Bermuda hay	55.0
Heifer Grain*	45.0
Composition	
Dry Matter (% of as fed)	95.5
Crude Protein	10.0
Available Protein	9.5
Adjusted Crude Protein	10.0
ADF	32.3
Lignin	4.4
NFC	22.5
Starch	8.4
ESC (Simple Sugars)	6.6
Crude Fat	2.2
Ash	7.17
TDN	61
NE_m (Mcal/kg)	1.25
NE_g (Mcal/kg)	0.68
Calcium	0.42
Phosphorus	0.30
Magnesium	0.32
Potassium	1.62
Sodium	0.134
PPM Iron	194
PPM Zinc	51
PPM Copper	12
PPM Manganese	93
PPM Molybdenum	<1
Sulfur	0.39
Chloride Ion	0.61

*511.8 g/kg dry rolled corn, 41.1 g/kg molasses, 211.2 g/kg dry rolled oats, 210.6 g/kg soybean meal, 2.8 g/kg Dical, 8.5 g/kg min./vit. pack, 2.8 g/kg salt, 11.2 g/kg limestone

Table 5. Chute temperament scoring system adopted from Grandin et al. (1995).

Score	Chute Temperament Definition
1	Calm, no movement
2	Restless shifting
3	Head throwing, squirming and occasionally shaking the squeeze chute
4	Violently and continually shaking the squeeze chute

Table 6. Chute exit scoring system adopted from Lanier and Grandin (2003).

Score	Chute Exit Definition
1	Walk
2	Trot
3	Run
4	Jump

Table 7. Pen temperament scoring system adopted from Hammond et al. (1996).

Score	Pen Temperament Definition
1	Non-aggressive (docile): Animal walks slowly, observer can approach closely, and animal is not excited by humans or facilities
2	Slightly aggressive: Animal walks quickly or trots away, carries head up at attention, and maintains distance as human approaches
3	Moderately aggressive: Animal trots or runs along fences, carries head high and is aware of humans, will move quickly as humans move closer, and commonly separates themselves from the group
4	Aggressive: Animal runs, stays in the back of group, carries head high and is very aware of humans, may run into fences and gates even with some distance, and will likely run along fences if alone in pen
5	Very aggressive: Animal is excited, runs into fences, runs over humans and anything else in its path, also referred to as "crazy"

Table 8. Least-squares means (n = 6) of heifer emissions and DMI by day within period for periods 2✚, 4✚, and 7◆.

		Period					
		2		4		7	
Item	Day	LS Mean	SE	LS Mean	SE	LS Mean	SE
CH_4 (L/hr)	1	9.8[a]	0.347	9.3[a]	0.296	7.4[b]	0.439
	2	9.5[a]	0.271	9.9[a]	0.275	8.0[b]	0.420
	3	10.1[a]	0.274	9.3[b]	0.284	8.7[b]	0.473
CO_2 (L/hr)	1	160.7[a]	4.234	151.9[b]	3.895	131.5[c]	4.785
	2	145.6[a]	3.843	151.3[b]	3.836	132.8[c]	4.663
	3	147.1[a]	3.858	150.8[a]	3.905	133.7[b]	5.002
O_2 (L/hr)	1	149.8[a]	4.531	145.3[a]	4.251	126.0[b]	4.995
	2	136.8[a]	4.209	143.5[b]	4.204	124.6[c]	4.891
	3	137.2[a]	4.221	143.0[b]	4.260	124.7[c]	5.180
DMI (kg)	1	10.2[a]	0.536	10.1[a]	0.536	8.4[b]	0.536
	2	8.9[a]	0.536	9.5[a]	0.536	8.4[a]	0.536
	3	8.6[a]	0.536	10.0[a]	0.536	8.3[a]	0.536
CH_4 (g/kg DMI)	1	17.1[a]	0.514	17.1[a]	0.531	16.2[a]	0.511
	2	17.8[a]	0.512	18.2[a]	0.517	16.9[b]	0.511
	3	19.3[a]	0.511	17.0[b]	0.511	18.0[b]	0.512

[a, b, c] Least-squares means within row without common superscript letters differ ($P < 0.05$).

✚For gas measurement periods 2 and 4, animals remained on *ad libitum* feed intake.

◆For the gas measurement period 7, feed was restricted to 2% of body weight on a dry matter basis.

Table 9. The least-squares means (n = 6) of temperature-humidity index (THI) and respiration rates for each period[✚].

Period	THI LS Mean	SE	Respiration Rate LS Mean	SE
1	46.4	1.234	28	0.567
2	62.3	1.834	35	1.041
3	60.1	1.234	29	0.444
4	67.7	1.834	36	1.083
5	62.2	1.263	33	0.498
6	62.3	1.681	32	0.911
7	66.7	1.834	33	1.074

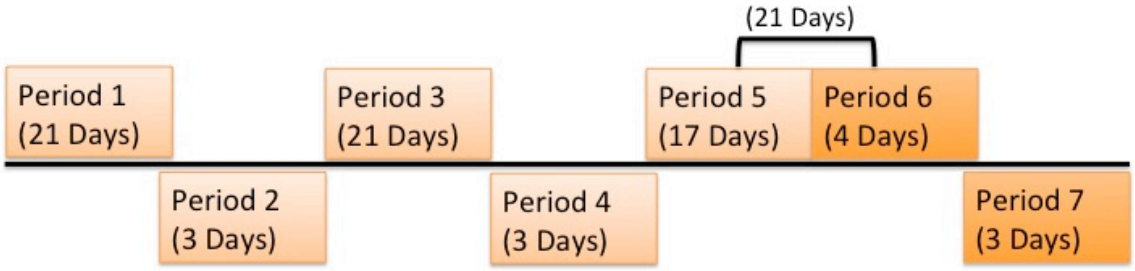
[✚] For periods 1, 3, 5, and 6, heifers were fed in individual pens. For gas measurement periods 2 and 4, animals remained on *ad libitum* feed intake, and for gas measurement period 7, feed was restricted to 2% of body weight on a dry matter basis.

Table 10. The differences of least-squares means of temperature-humidity index (THI) and respiration rate for each period[✚].

Period	Period for Comparison	THI Estimated Difference	SE	*P*-Value	Respiration Rate Estimated Difference	SE	*P*-Value
1	2	-15.93	1.566	<.0001	4.40	1.269	0.0104
1	4	-21.30	1.566	<.0001	-2.43	1.431	0.616
1	5	-15.83	0.828	<.0001	-3.00	1.423	0.3509
1	3	-13.69	0.783	<.0001	-0.13	1.102	1
1	6	-15.94	1.384	<.0001	3.38	1.098	0.0357
2	4	-5.38	2.072	0.1302	0.64	1.339	0.9991
2	5	0.10	1.589	1	-6.83	1.198	<.0001
2	3	2.24	1.566	0.787	-7.39	1.286	<.0001
2	6	-0.01	1.938	1	-4.53	0.773	<.0001
3	6	-2.24	1.384	0.669	-1.02	0.703	0.7772
4	5	5.47	1.589	0.0112	-3.76	1.087	0.0107
4	3	7.61	1.566	<.0001	-0.57	1.434	0.9997
4	6	5.37	1.938	0.0844	2.30	1.092	0.3479
5	3	2.14	0.828	0.1332	5.81	1.078	<.0001
5	6	-0.10	1.410	1	3.07	1.331	0.2429
7	1	20.35	1.566	<.0001	2.87	1.106	0.1306
7	2	4.43	2.072	0.3335	6.38	1.105	<.0001
7	4	-0.95	2.072	0.9993	3.64	1.343	0.0988
7	5	4.52	1.589	0.0692	3.51	0.573	<.0001
7	3	6.66	1.566	0.0005	0.77	0.969	0.9857
7	6	4.42	1.938	0.2566	-2.74	0.954	0.0637

[✚] For periods 1, 3, 5, and 6, heifers were fed in individual pens. For gas measurement periods 2 and 4, animals remained on *ad libitum* feed intake, and for gas measurement period 7, feed was restricted to 2% of body weight on a dry matter basis.

Figure 3. Periods 1 – 7[✚] presented as a timeline.



[✚] For periods 1, 3, and 5 heifers were fed *ad libitum* in individual pens. For gas measurement periods 2 and 4, animals remained on *ad libitum* feed intake. For period 6, feed was restricted to 2% of body weight on a dry matter basis, and heifers remained in their individual pens. For gas measurement period 7, heifers remained on restricted feed intake.

Figure 4. A diagram depicting an overview of the head box system.

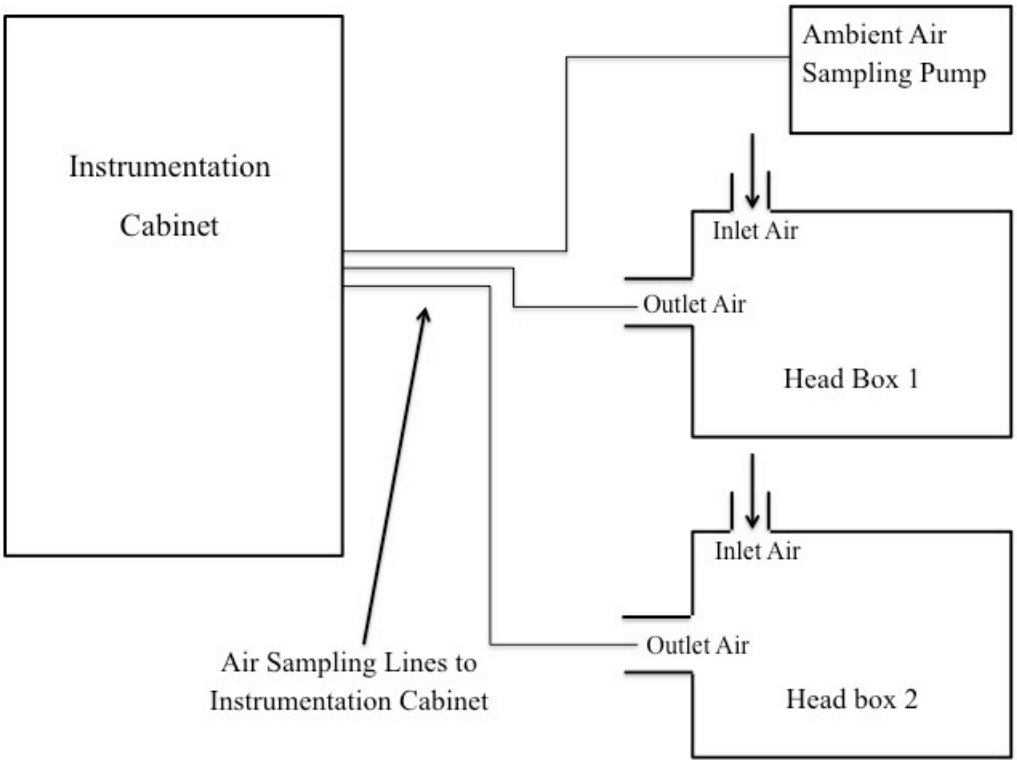


Figure 5. Two empty head boxes placed side-by-side and attached to metabolism stalls.

Figure 6. An example of a heifer eating in the head box during a gas measurement period.

Figure 7. The instrumentation cabinet containing the FlowKits (A), SS-4 sub-sampler pump (B), RH-300 water vapor analyzer (C), CA-10 carbon dioxide analyzer (D), MA-10 methane analyzer (E), FC-10 oxygen analyzer (F), computer (G), and A/C unit (H) located adjacent to the head boxes.

Figure 8. A screenshot of a recovery rate test injection showing the start and end of CO_2 injection into head box 1. The first horizontal line indicates when the CO_2 injection began, and the second horizontal line indicates when the CO_2 injection was stopped.

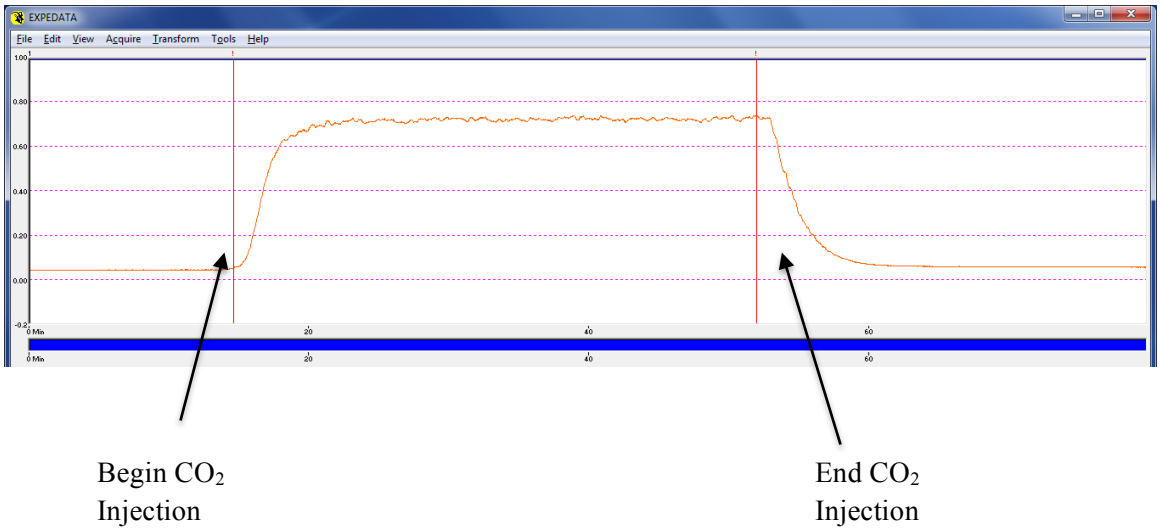


Figure 9. Activity logger attached to the right hind leg of a heifer in her individual feeding pen during the study.

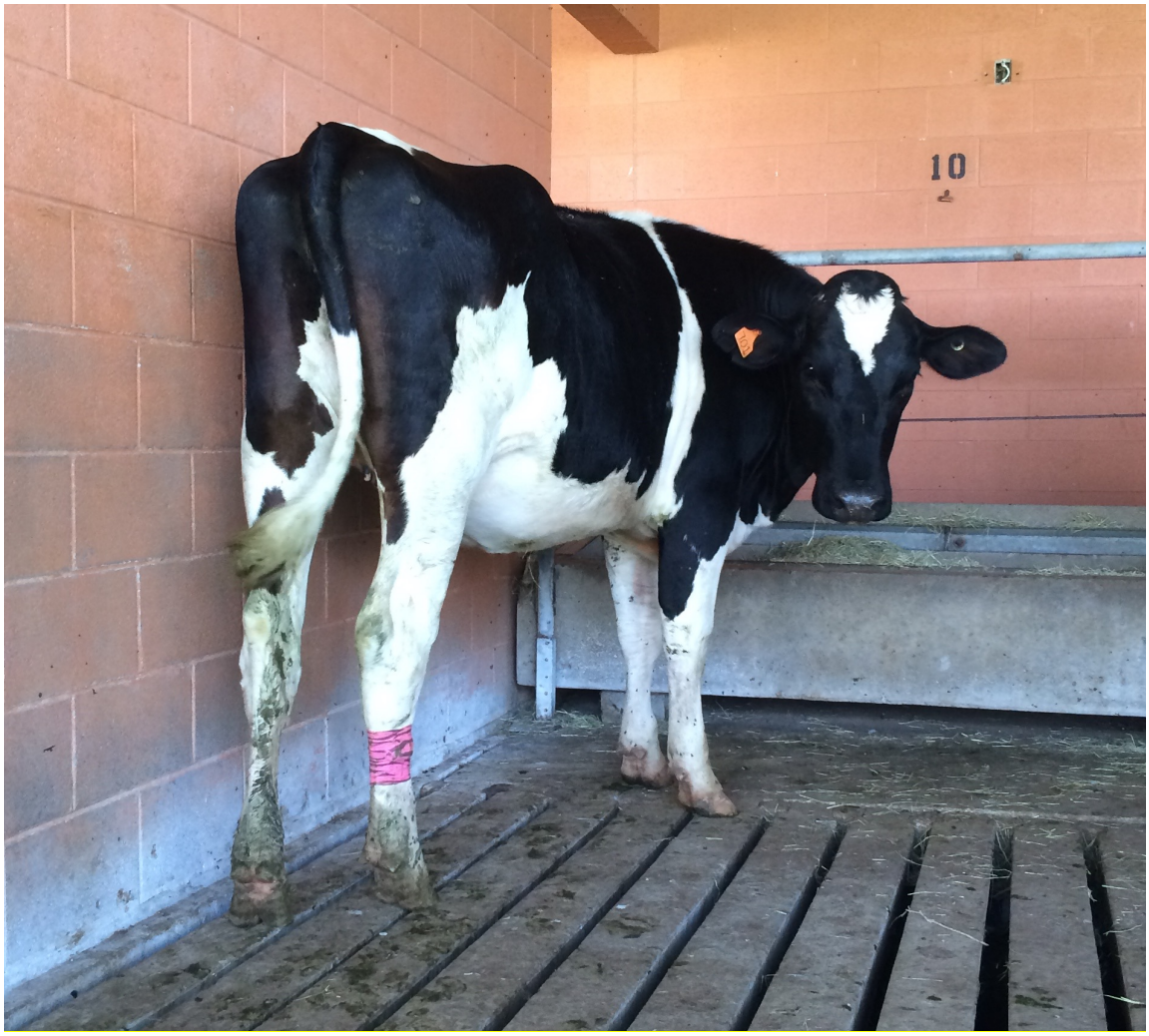
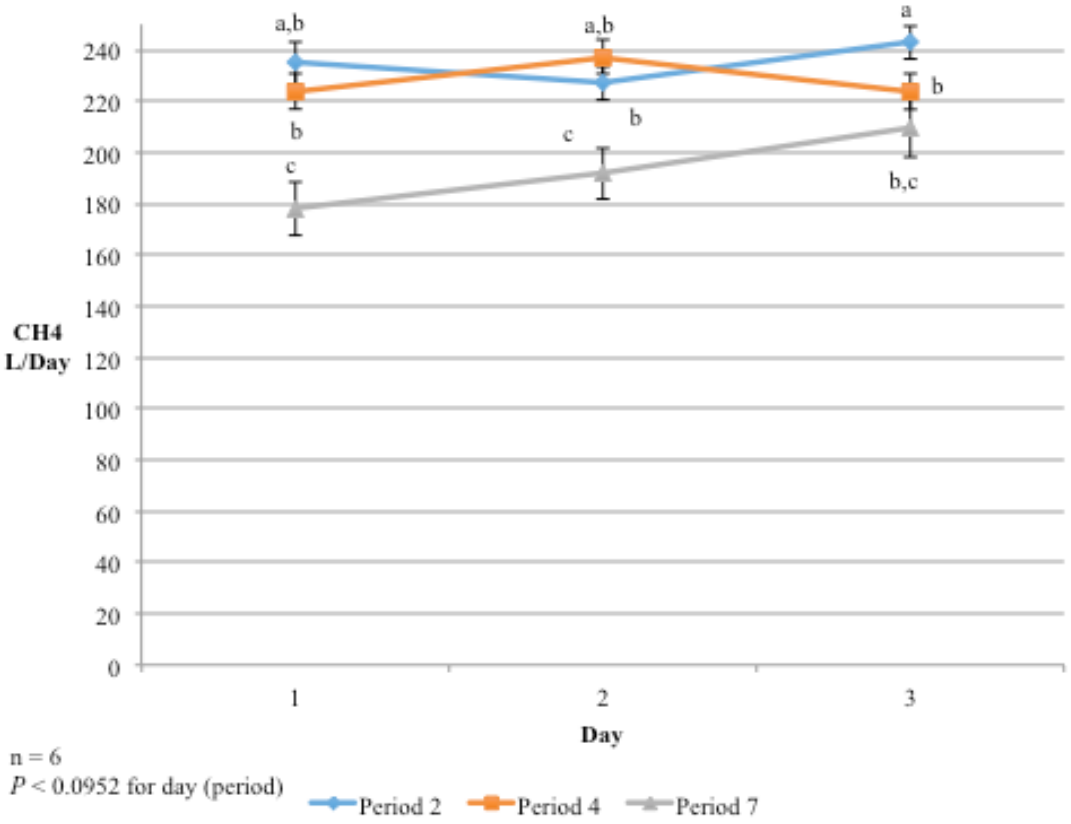


Figure 10. Least-squares means of CH_4 per heifer for day within period for periods 2✚, 4✚, and 7◆.

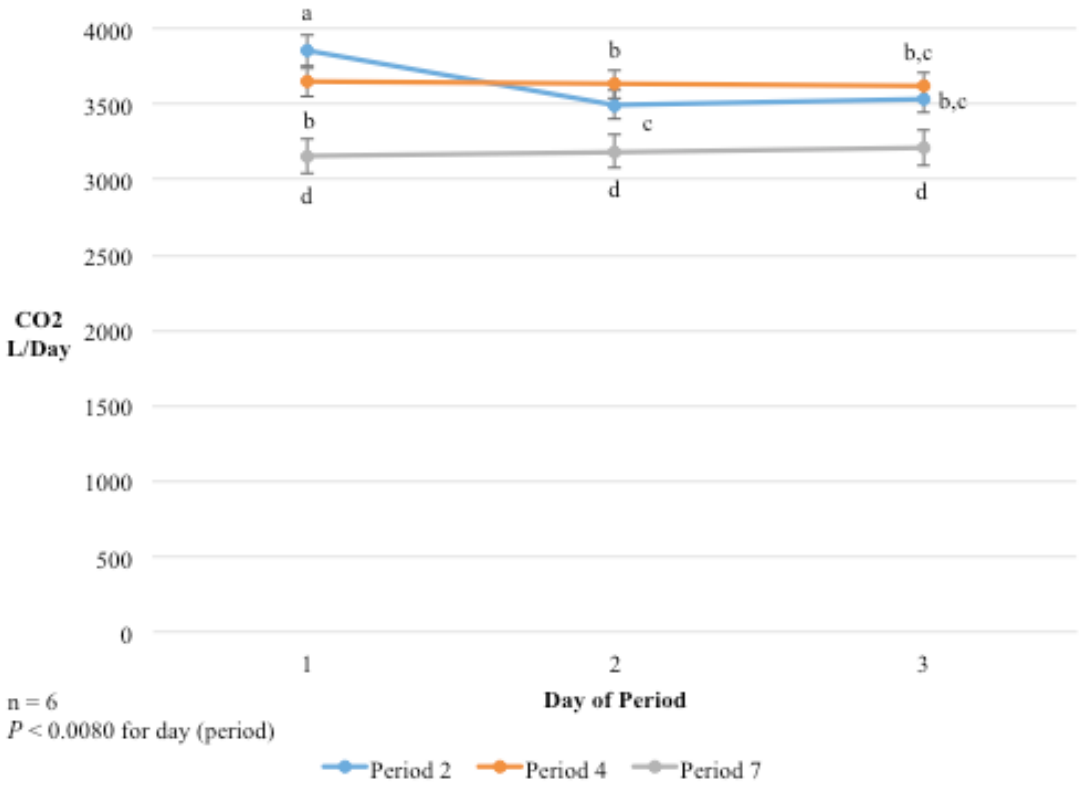


[a, b, c] Least-squares means without common superscripts differ ($P < 0.05$).

✚For gas measurement periods 2 and 4, animals remained on *ad libitum* feed intake.

◆For the gas measurement period 7, feed was restricted to 2% of body weight on a dry matter basis.

Figure 11. Least-squares means of CO_2 per heifer for day within period for periods 2✚, 4✚, and 7◆.

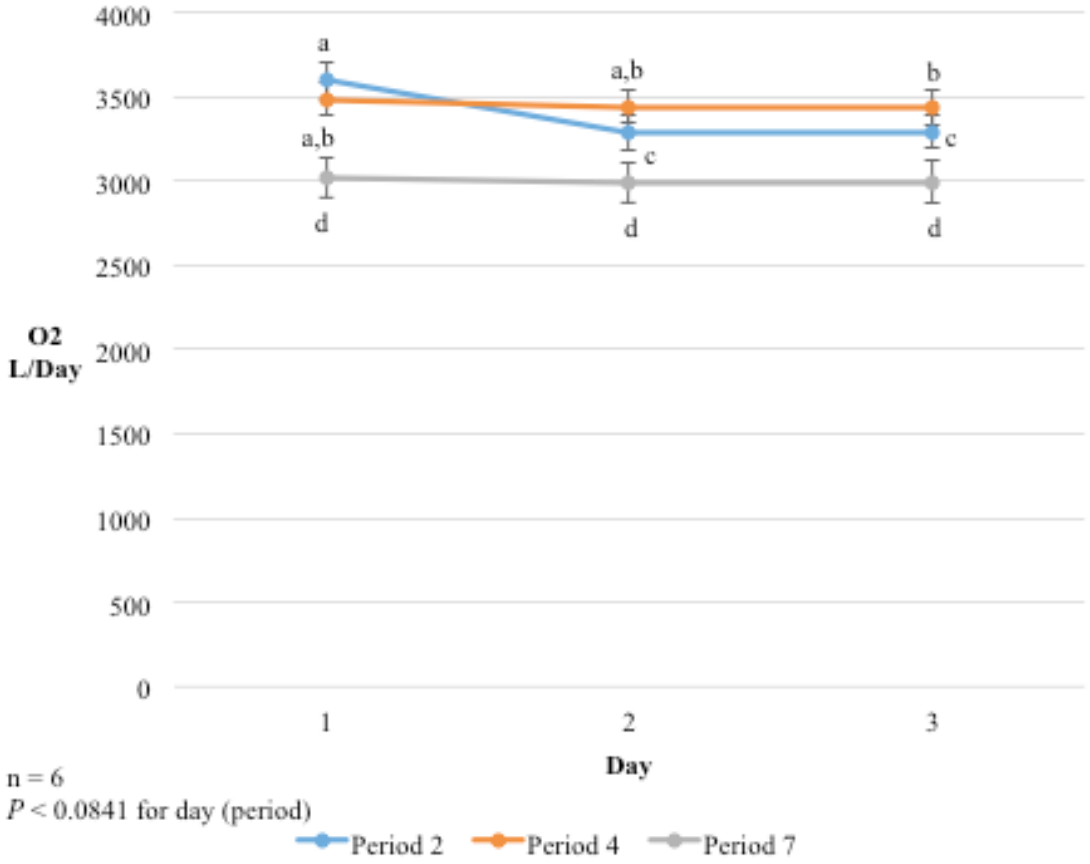


[a, b, c, d] Least-squares means without common superscripts differ ($P < 0.05$).

✚For gas measurement periods 2 and 4, animals remained on *ad libitum* feed intake.

◆For the gas measurement period 7, feed was restricted to 2% of body weight on a dry matter basis.

Figure 12. Least-squares means of O_2 per heifer for day within period for periods 2✚, 4✚, and 7◆.

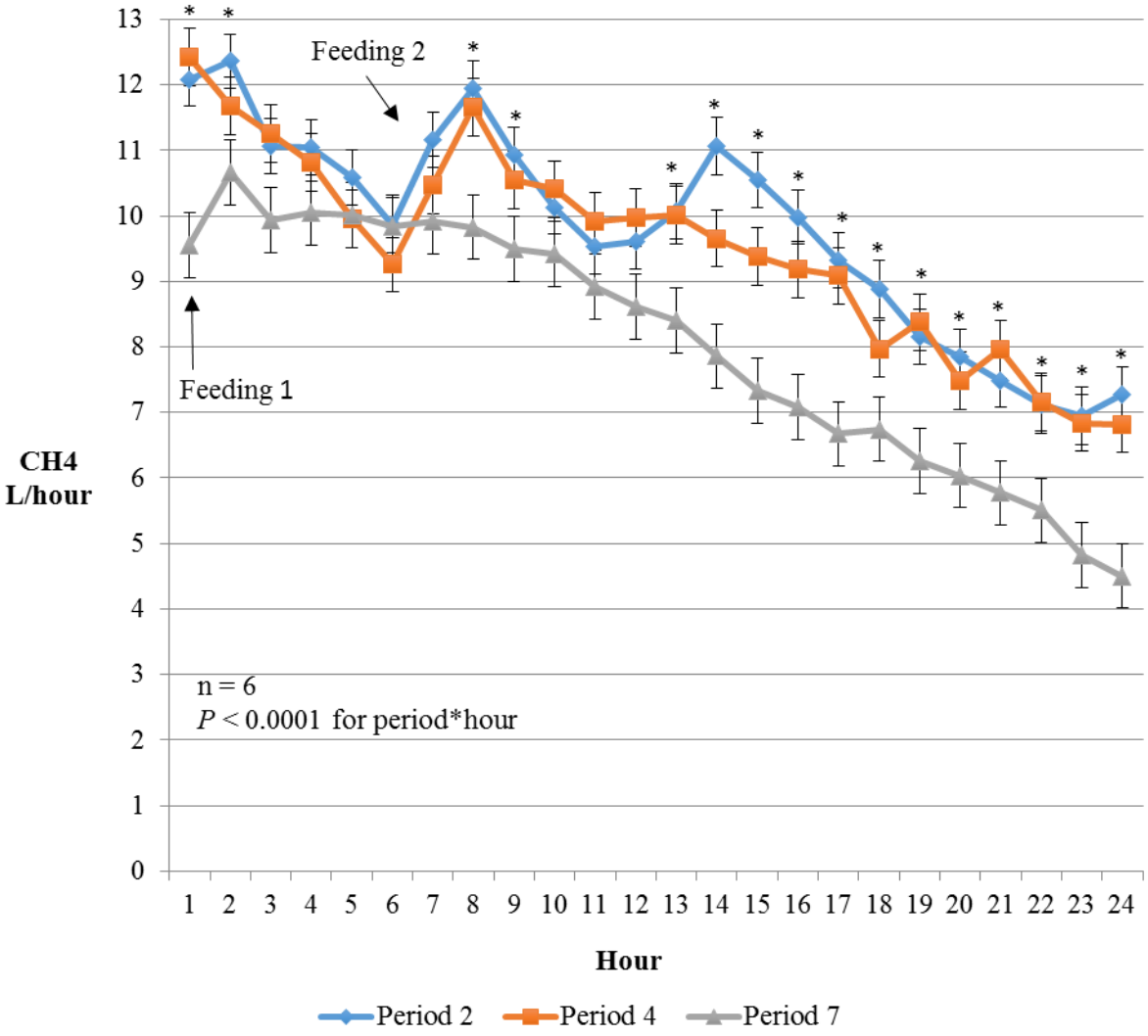


[a, b, c, d] Least-squares means without common superscripts differ ($P < 0.05$).

✚For gas measurement periods 2 and 4, animals remained on *ad libitum* feed intake.

◆For the gas measurement period 7, feed was restricted to 2% of body weight on a dry matter basis.

Figure 13. Least-squares means of period by hour interaction for CH_4 (L/hour). Heifers were fed twice daily in periods 2✚ and 4✚ at hour 1 and 7, and were fed once daily in period 7◆ at hour 1.

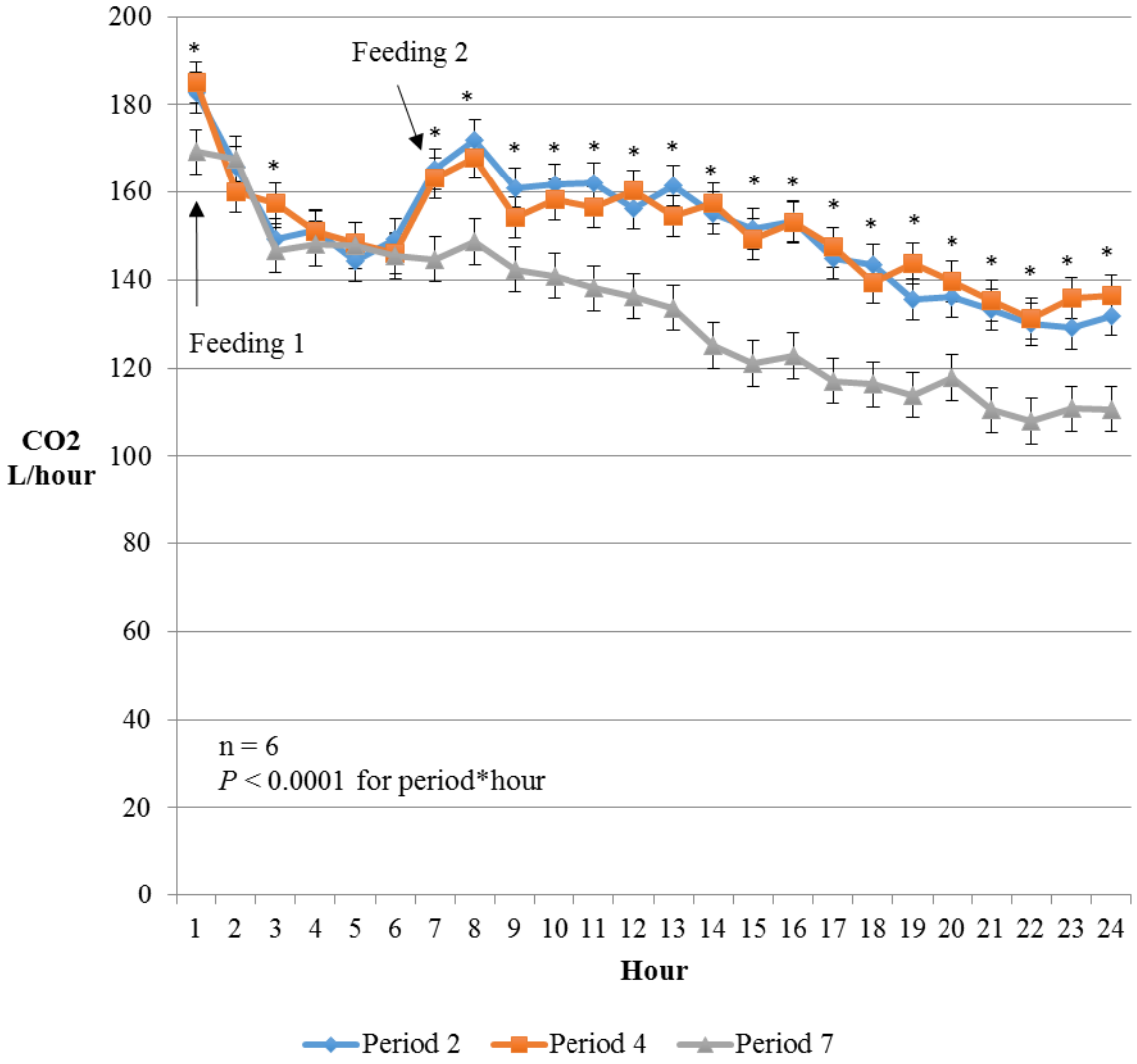


* Least-squares means within hour differ ($P < 0.05$).

✚For gas measurement periods 2 and 4, animals remained on *ad libitum* feed intake.

◆For the gas measurement period 7, feed was restricted to 2% of body weight on a dry matter basis.

Figure 14. Least-squares means of period by hour interaction for CO_2 (L/hour). Heifers were fed twice daily in periods 2✚ and 4✚ at hour 1 and 7, and were fed once daily in period 7◆ at hour 1.

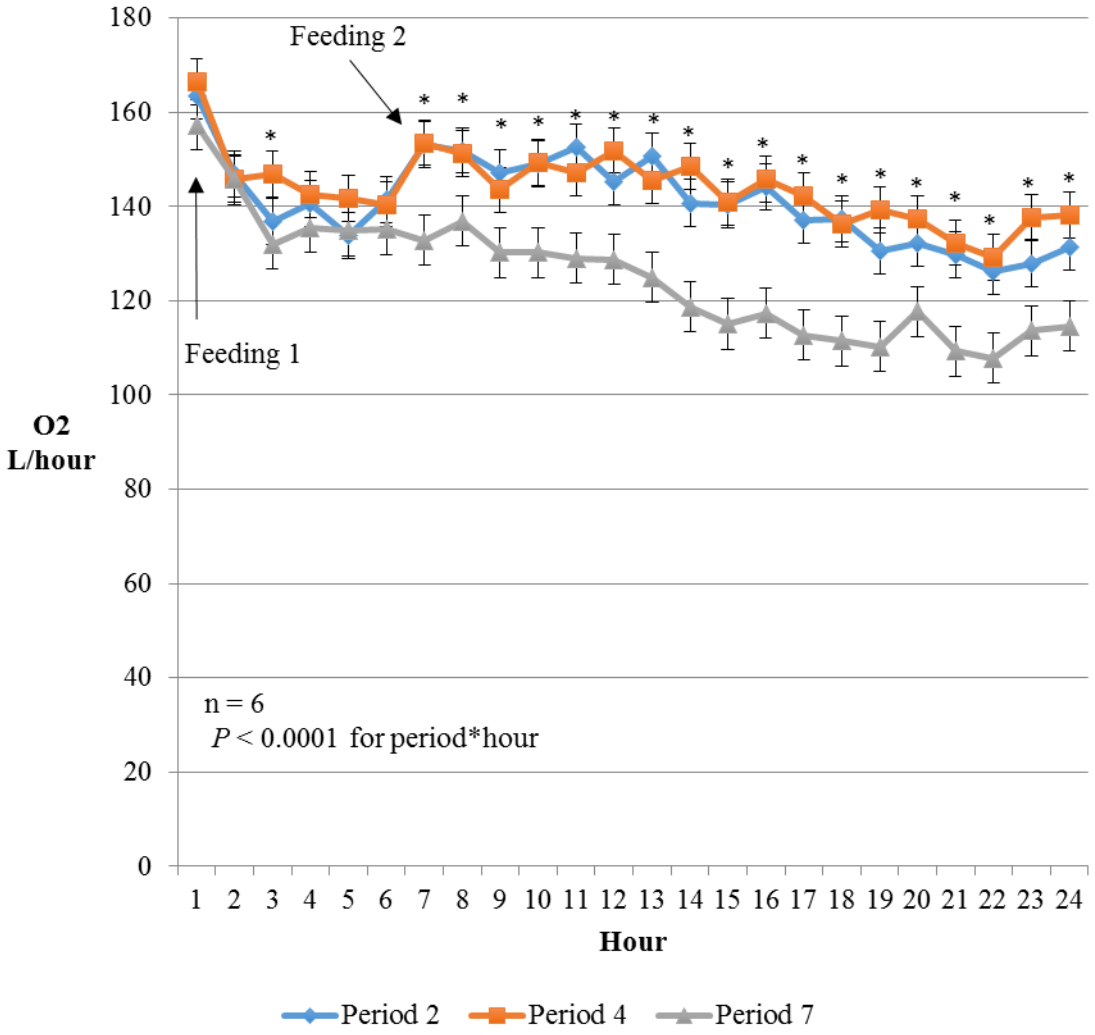


* Least-squares means within hour differ ($P < 0.05$).

✚For gas measurement periods 2 and 4, animals remained on *ad libitum* feed intake.

◆For the gas measurement period 7, feed was restricted to 2% of body weight on a dry matter basis.

Figure 15. Least-squares means of period by hour interaction for O_2 (L/hour). Heifers were fed twice daily in periods 2✚ and 4✚ at hour 1 and 7, and were fed once daily in period 7◆ at hour 1.

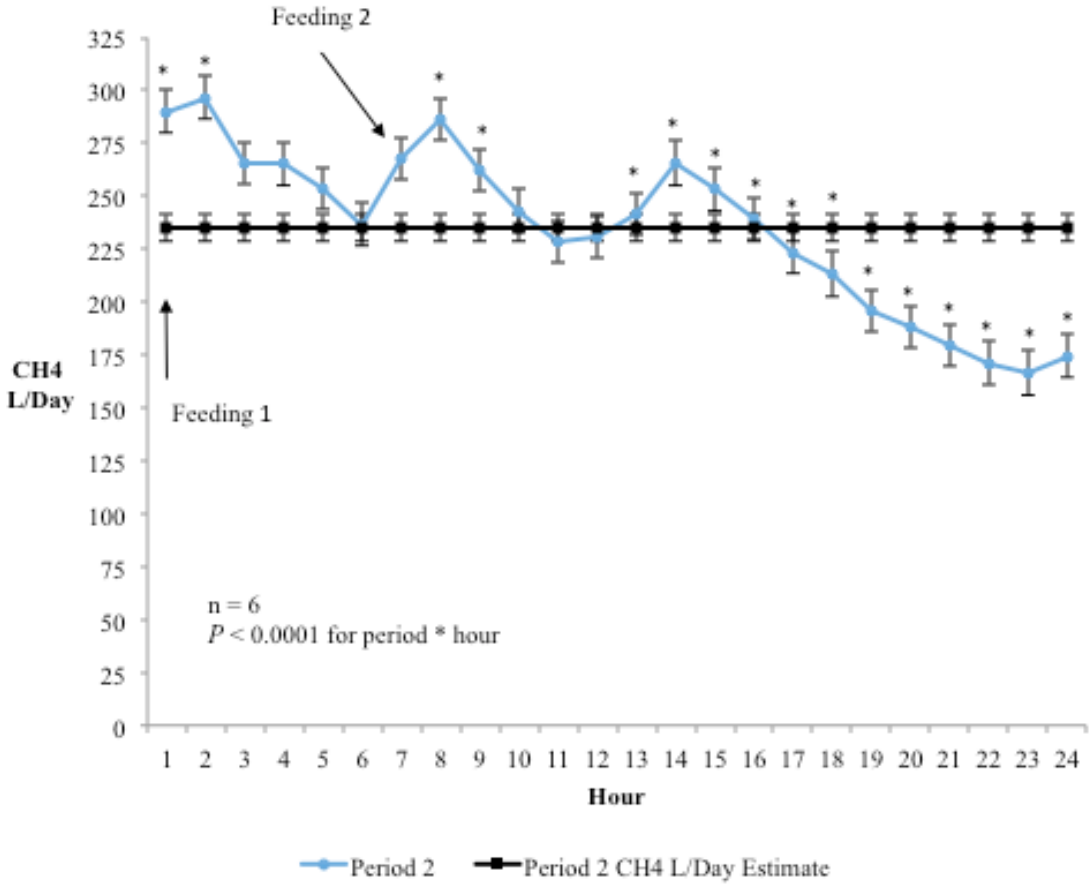


* Least-squares means within hour differ ($P < 0.05$).

✚For gas measurement periods 2 and 4, animals remained on *ad libitum* feed intake.

◆For the gas measurement period 7, feed was restricted to 2% of body weight on a dry matter basis.

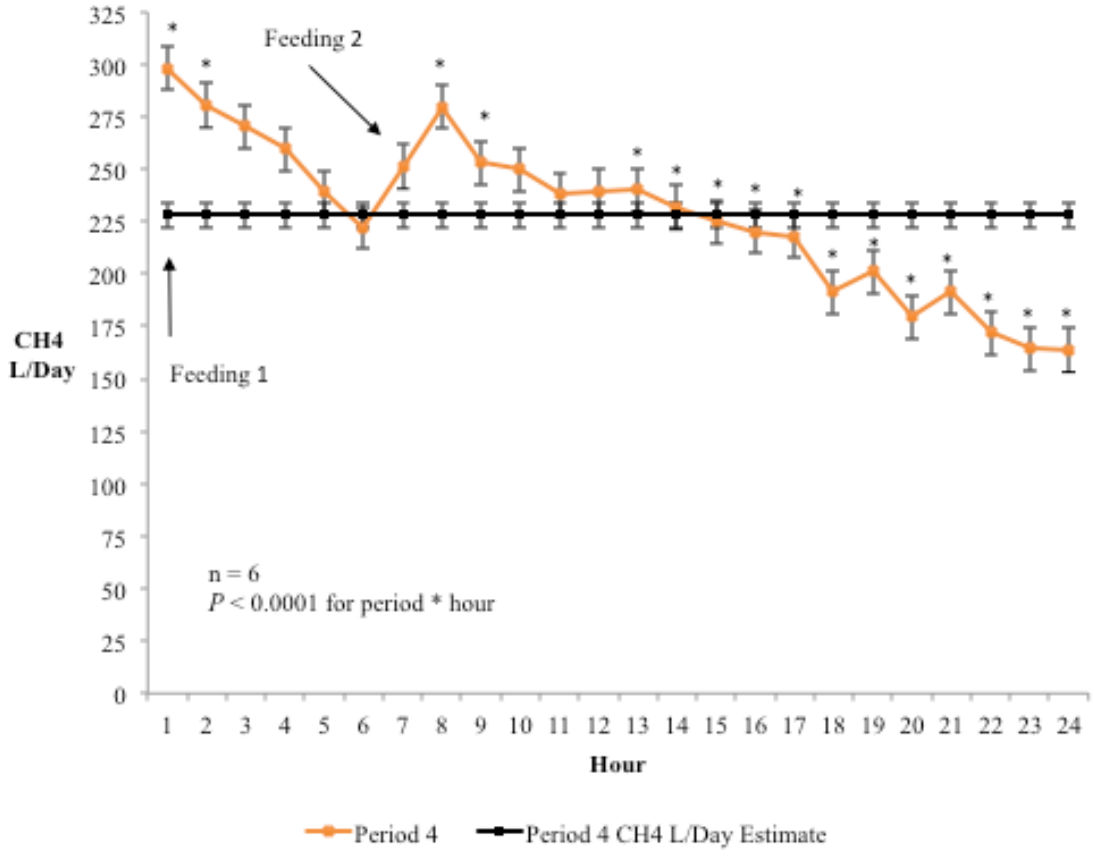
Figure 16. Least-squares means estimates of period by hour interaction for CH_4 (L/Day) for period 2 presented with the least-squares means estimate of CH_4 (L/Day) for period 2. Heifers were fed twice daily in period 2✚ at hour 1 and 7.



* Least-squares means within hour differ ($P < 0.05$) across periods 2, 4, and 7.

✚For gas measurement period 2, animals remained on *ad libitum* feed intake.

Figure 17. Least-squares means estimates of period by hour interaction for CH_4 (L/Day) for period 4 presented with the least-squares means estimate of CH_4 (L/Day) for period 4. Heifers were fed twice daily in period 4✚ at hour 1 and 7.

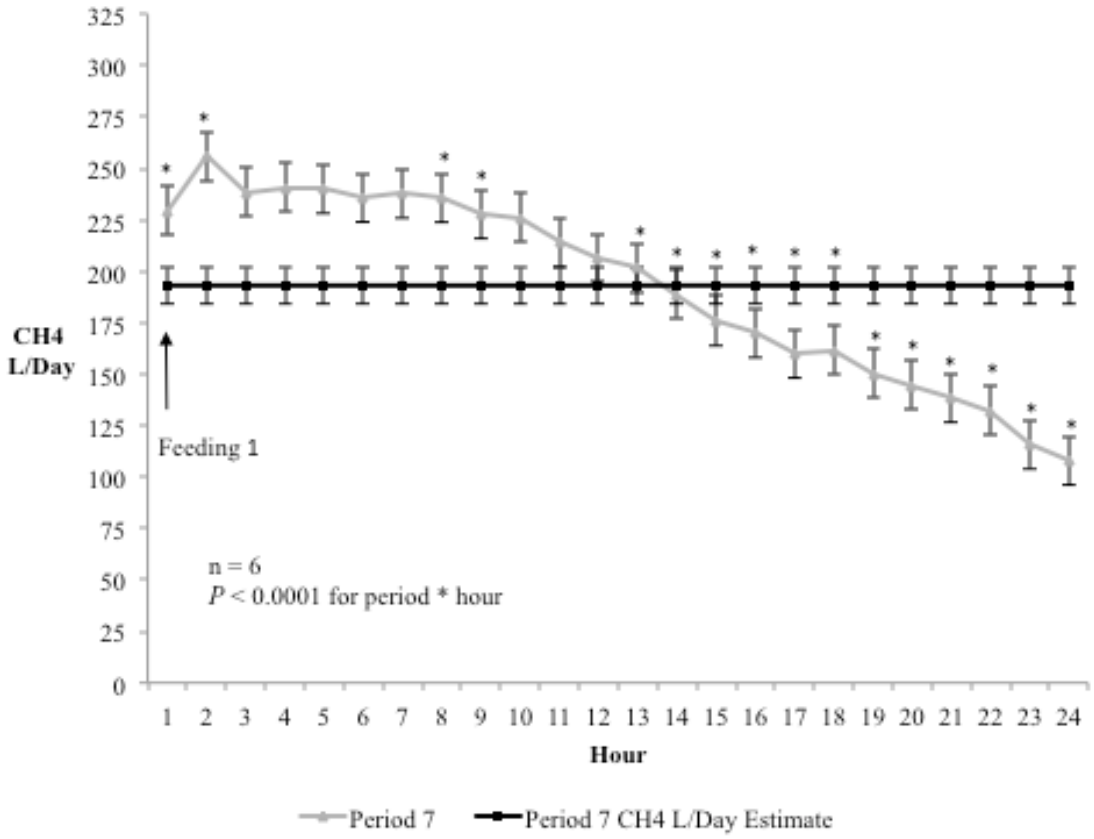


* Least-squares means within hour differ ($P < 0.05$) across periods 2, 4, and 7.

✚For gas measurement period 4, animals remained on *ad libitum* feed intake.

◆For the gas measurement period 7, feed was restricted to 2% of body weight on a dry matter basis.

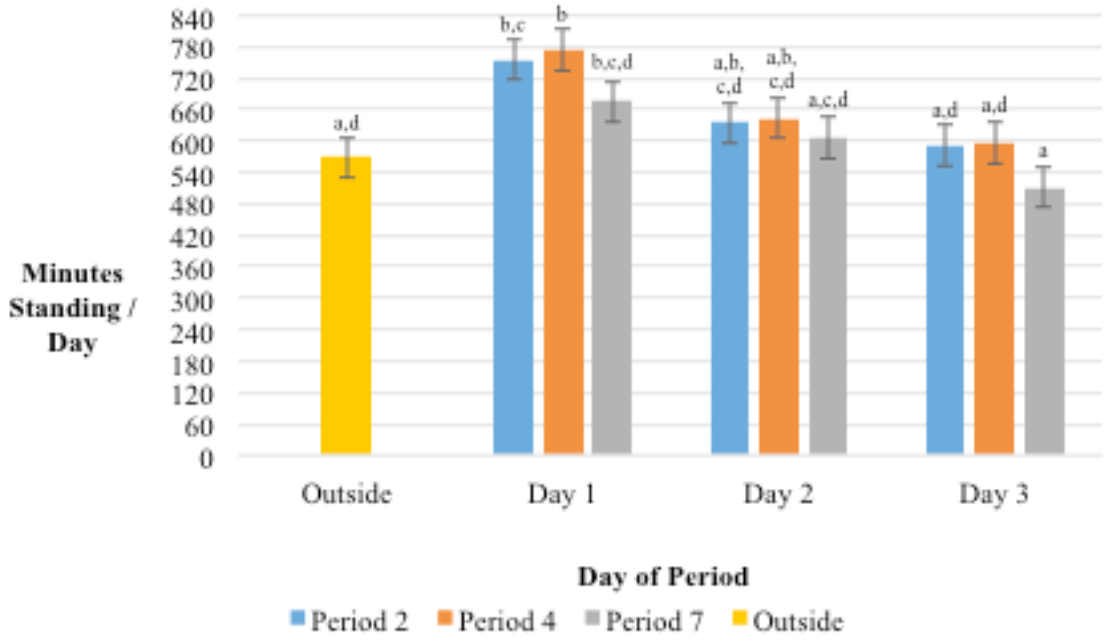
Figure 18. Least-squares means estimates of period by hour interaction for CH_4 (L/Day) for period 7 presented with the least-squares means estimate of CH_4 (L/Day) for period 7. Heifers were fed once daily in period 7✚ at hour 1.



* Least-squares means within hour differ ($P < 0.05$) across periods 2, 4, and 7.

✚For gas measurement period 7, feed was restricted to 2% of body weight on a dry matter basis.

Figure 19. Least-squares means (n = 6) of cattle standing duration for each day of periods 2✚, 4✚, and 7◆ shown with the mean standing time of periods 1, 3, 5, and 6 labeled as "outside".

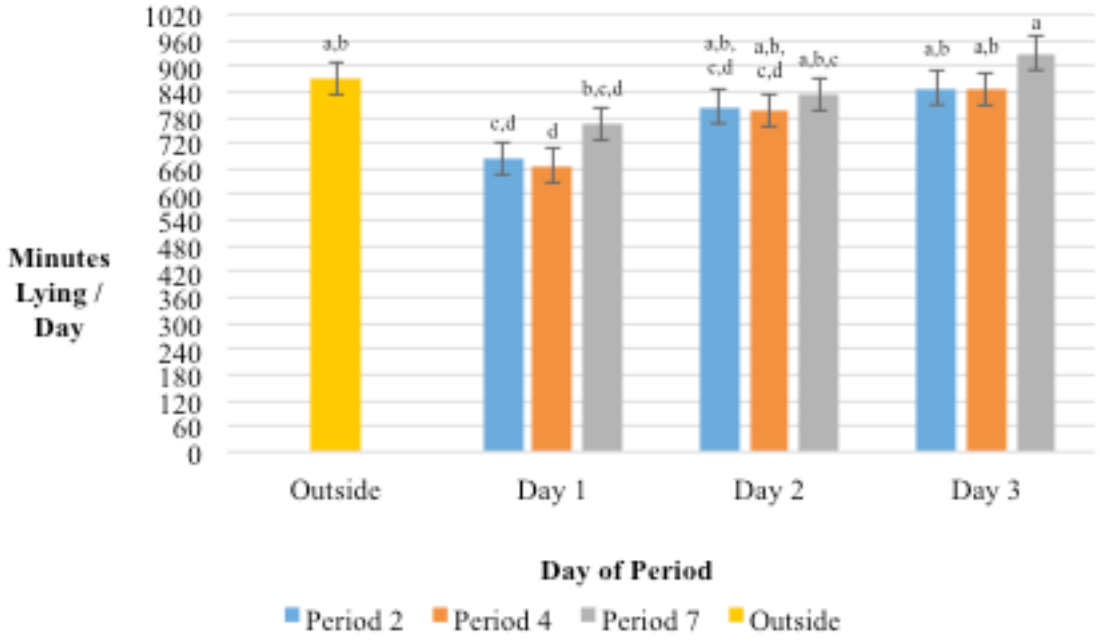


[a, b, c, d] Least-squares means without common superscript letters differ ($P < 0.05$).

✚For gas measurement periods 2 and 4, animals remained on *ad libitum* feed intake.

◆For the gas measurement period 7, feed was restricted to 2% of body weight on a dry matter basis.

Figure 20. Least-squares means (n = 6) of cattle lying duration for each day of periods 2✚, 4✚, and 7◆ shown with the mean lying time of periods 1, 3, 5, and 6 labeled "outside".

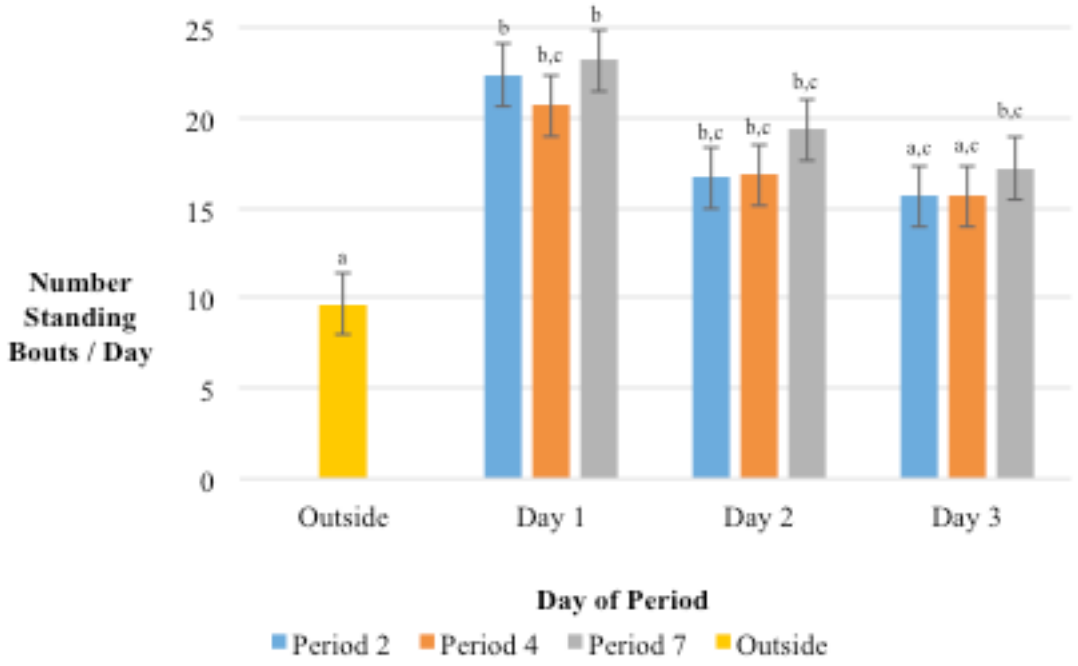


[a, b, c, d] Least-squares means without common superscript letters differ ($P < 0.05$).

✚For gas measurement periods 2 and 4, animals remained on *ad libitum* feed intake.

◆For the gas measurement period 7, feed was restricted to 2% of body weight on a dry matter basis.

Figure 21. Least-squares means (n = 6) of cattle standing bouts for each day of periods 2✚, 4✚, and 7◆ shown with the mean number of standing bouts per day of periods 1, 3, 5, and 6 labeled "outside".

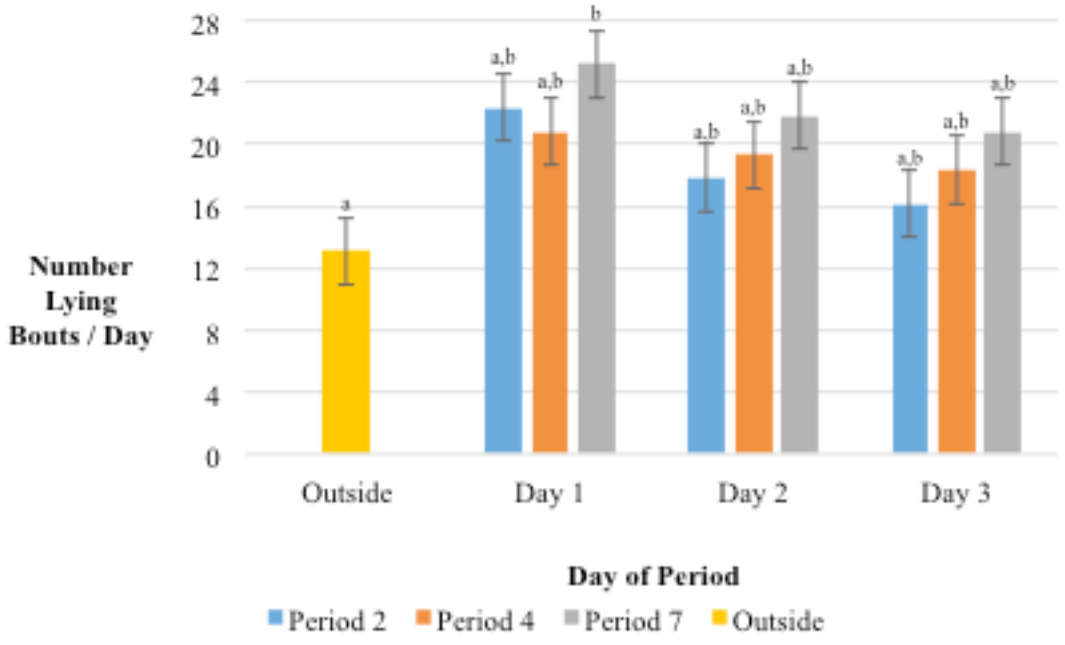


[a, b, c] Least-squares means without common superscript letters differ ($P < 0.05$).

✚For gas measurement periods 2 and 4, animals remained on *ad libitum* feed intake.

◆For the gas measurement period 7, feed was restricted to 2% of body weight on a dry matter basis.

Figure 22. Least-squares means (n = 6) of cattle lying bouts for each day of periods 2✚, 4✚, and 7◆ shown with the mean number of lying bouts per day of periods 1, 3, 5, and 6 labeled "outside".

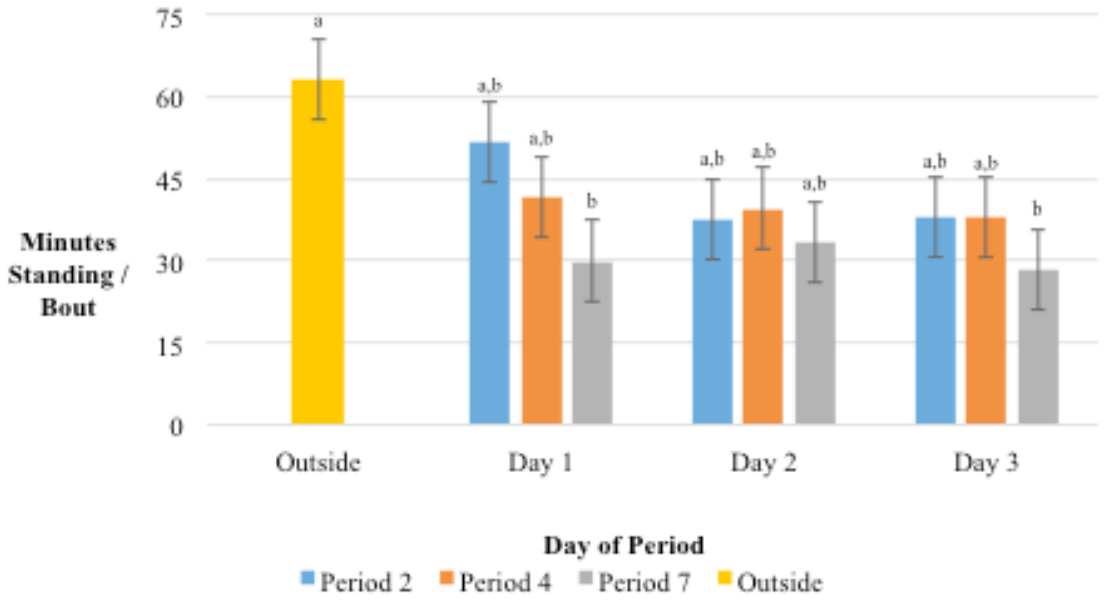


[a, b] Least-squares means without common superscript letters differ ($P < 0.05$).

✚For gas measurement periods 2 and 4, animals remained on *ad libitum* feed intake.

◆For the gas measurement period 7, feed was restricted to 2% of body weight on a dry matter basis.

Figure 23. Least-squares means (n = 6) of cattle standing duration per bout for each day of periods 2✚, 4✚, and 7◆ shown with the mean standing duration per bout of periods 1, 3, 5, and 6 labeled "outside".

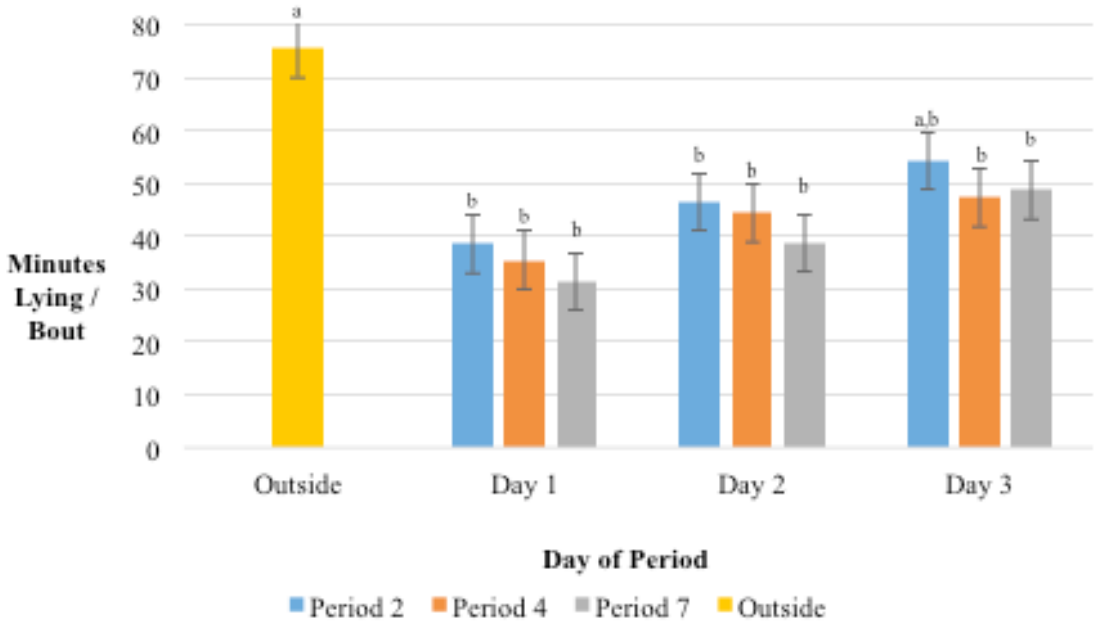


[a, b] Least-squares means without common superscript letters differ ($P < 0.05$).

✚For gas measurement periods 2 and 4, animals remained on *ad libitum* feed intake.

◆For the gas measurement period 7, feed was restricted to 2% of body weight on a dry matter basis.

Figure 24. Least-squares means (n = 6) of cattle lying duration per bout for each day of periods 2✚, 4✚, and 7◆ shown with the mean lying duration per bout of periods 1, 3, 5, and 6 labeled "outside".

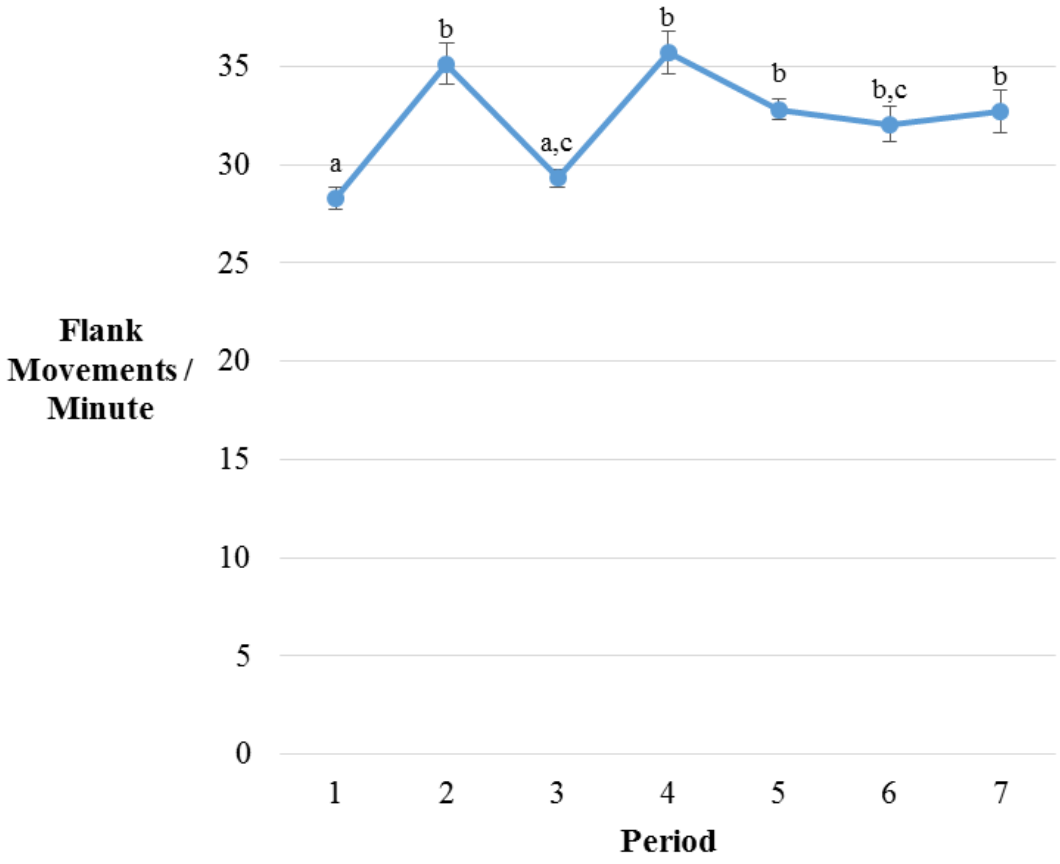


[a, b] Least-squares means without common superscript letters differ ($P < 0.05$).

✚For gas measurement periods 2 and 4, animals remained on *ad libitum* feed intake.

◆For the gas measurement period 7, feed was restricted to 2% of body weight on a dry matter basis.

Figure 25. The least-squares means of respiration rate (flank movements / minute) for each period✚.



[a, b, c] Least-squares means without common superscript letters differ ($P < 0.05$).

✚ For periods 1, 3, 5, and 6, heifers were fed in individual pens. For gas measurement periods 2 and 4, animals remained on *ad libitum* feed intake, and for gas measurement period 7, feed was restricted to 2% of body weight on a dry matter basis.

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