DESCRIPTION AND COMMISSIONING OF A NOVEL SWINE CALORIMETER UNIT TO CALCULATE HEAT PRODUCTION AND NET ENERGY IN GROUP-HOUSED PIGS

BY

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THESIS

Submitted in partial fulfillment of the requirements
for the degree of Master of Science in Animal Sciences
in the Graduate College of the
University of Illinois at Urbana-Champaign, 2020

Urbana, Illinois

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ABSTRACT

Values for digestible energy (**DE**), metabolizable energy (**ME**) and net energy (**NE**) are used to develop prediction equations for diet formulation, usually calculated from data for individually housed pigs. However, pigs in commercial conditions are usually housed in groups and allowed *ad-libitum* feed intake. It is possible therefore that, energy values in group-housed pigs are different from values obtained in individually housed pigs. The Swine Calorimeter Unit (**SCU**) at the University of Illinois has been constructed to obtain energy values of diets and feed ingredients in group-housed pigs that are allowed *ad-libitum* access to feed. The SCU contains 6 calorimeter chambers with a capacity to hold 4 to 10 growing-finishing pigs in each chamber. The SCU allows for calculation of DE, ME, and NE of diets because of total, but separate, collection of feces and urine, and measurements of gas exchange in the chambers. Two experiments were conducted as part of the commissioning of the SCU. The first experiment had the objective of testing the hypothesis that there are no differences in the estimated values for total heat production (**THP**) and NE among the 6-calorimeter chambers if all environmental and dietary conditions are similar. Results indicated that the NE of the diet had a coefficient of variation (**CV**) of 4.2 % among chambers. The second experiment tested the hypothesis that a greater protein concentration in the diets may result in a greater THP and, therefore, a lower NE value. Results indicated that there were no differences in the NE values between the 2 diets. The energetic loss associated with deamination of excess AA and, urea synthesis may be less than previously believed. Therefore, more research is needed to demonstrate how dietary protein affects THP and NE values of group-housed pigs.

Keywords: calorimeter, group housing, net energy, pigs.

ACKNOWLEDGEMENTS

First, I would like to thank my advisor, Dr. H. H. Stein, who gave me the opportunity to join his team. I am forever grateful for his confidence in me to be involved in this great new project (the Swine Calorimeter Unit). I am thankful for him trusting me and giving me the opportunity to continue my professional development. It was an honor to be part of the Stein Monogastric Nutrition Laboratory.

I want to thank the Guerrero-Casas family, who trusted me from the beginning and helped me come to the University of Illinois and pursue my dreams. Thank you Dr. "profe" Casas for your unconditional support and advice, and for your guidance and company through this journey. I hope that I can show others the same type of support that you showed me.

I also want to express my gratitude to all the members of the Stein lab. In my time here, I quickly realized that research is a team job and I would not have been able to do it without you all. In the future, I hope that I find more people like you to work with. I would like to extend a special thanks to the "guys," who have been my brothers here. You have been great company and support. Thank you, *homies*!

I want to thank my family, my driving force, for whom I want a better future. My parents, Imelda and Orlando, my siblings, Joha and Fito, and my little ones: my godchildren, Adeline and Mathias, and my nephew, Joaquin "coquito". You guys are my life. I will continue working hard to make you feel proud of me and help you as much as I can.

Last but not least, I want to extend my deepest gratitude to my co-worker, and a very important person in my life, Vanessa Lagos. Thank you for your unconditional support and advice. Thank you for always believing in me, not only during this master's degree program, but since the first time we met. I will always be grateful for you, Vane.

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CHAPTER 1: INTRODUCTION

Energy is the most expensive component of diets for pigs and the energy concentration in diets plays an important economical role in the swine industry (Kil et al., 2013). Therefore, energy requirements by pigs need to be determined with accuracy and need to be met in diet formulation. To estimate energy contributions from feed ingredients, the chemical composition needs to be known. Analysis of gross energy indicates the total amount of energy that a feed ingredient or a mixed diet contains, the digestible energy (**DE**) value shows the amount of energy that is absorbed by the animal, and the metabolizable energy (**ME**) indicates the amount of energy that is metabolized (Stewart, 2007). Systems based on DE and ME have been widely used in the feed industry. However, net energy (**NE**) of feed ingredients and diets may be more accurate because values for NE include the energy that was lost in the form of heat (Li et al., 2018). The most commonly used technique to determine NE is based on indirect calorimetry due to the lower complexity and greater accuracy of this procedure compared with other techniques such as the direct calorimetry method (Blaxter, 1989). The NE values have been mostly determined using individually housed pigs, but because pigs on commercial farms are usually housed in groups, it is believed that NE values from pigs housed in groups may be more accurate. The University of Illinois at Urbana-Champaign has, therefore, constructed a facility to determine NE in diets and feed ingredients fed to group-housed pigs. The commissioning phase of this facility has been concluded, and the facility is now ready for use. Therefore, the objective of the work included in this thesis was to describe the swine calorimeter unit at the University of Illinois and to provide examples of determination of NE values in the facility.

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CHAPTER 2: NET ENERGY DETERMINATION IN GROUP HOUSED PIGS USING A NOVEL CALORIMETER UNIT: REVIEW OF LITERATURE

INTRODUCTION

Energy is the most expensive component in a commercial swine diet (Kil et al., 2013), and supplying the correct amount of energy to pigs is one of the primary goals in swine feeding. To achieve this aim, energy requirements of the animals must be determined and the absorption and retention of nutrients from a given diet need to be known.

The general concept of nutritional energetics has been known since the middle of the 15th century (Johnson et al., 2003), and research in this area has contributed to an increased understanding of the energy flow from feed to animal. Since the establishment of the thermodynamic laws, it has been known that energy may be transformed from one form to other forms, and thus, if the intake of energy is known, it is possible to calculate the outcome. Antoine Laurent Lavoisier argued that "respiration is nothing but a slow combustion of carbon and hydrogen, comparable to a lamp or a lighted candle. Thus, the animal's breath burns substances and consume themselves" (West, 1994). Therefore, it may be inferred that the vital energy comes from the transformation of the energy in feed into biochemical energy used by the animal. To determine the total amount of energy contained in the feed, an adiabatic bomb calorimeter was developed in 1886 by the scientist Berthelot (Kopperl and Parascandola, 1971). This novel apparatus also allowed for determination of energy excreted in feces and urine, and energy balance studies could be performed. Lavoisier also conducted experiments to demonstrate the relationship between oxygen **(O_2)** in the air and the production of carbon dioxide (**CO_2**) and heat.

However, the ratio between the amount of O_2 and CO_2 production was first determined using an open-circuit calorimeter developed by the chemist Pettenkofer in 1862 (Heymsfield et al., 2016).

DETERMINATION OF ENERGY

Conventional energy partitioning began with determination of gross energy (**GE**) from the feed. This is calculated by the ignition of a feed sample using a bomb calorimeter (Kil et al., 2013). The energy in feces is also determined using this procedure, and this value is subtracted from the energy of the feed to determine digestible energy (**DE**) as described in the NRC (2012) according to the following equation [1]:

$$DE = [(GE - \text{energy in feces})] \qquad [1]$$

Metabolizable energy (**ME**) is calculated by subtracting the energy lost in urine and combustion gases such as methane from DE. However, usually the energy lost in combustion gases is ignored in pigs due to the low production of these gases by monogastric animals. Metabolizable energy is, therefore, calculated according to the following equation [2] (NRC, 2012):

$$ME = [(DE - \text{energy in urine})] \qquad [2]$$

Net energy (**NE**) is commonly known as the energy resulting from subtraction of the energy losses in heat increment from ME (NRC, 2012). These energy losses are mainly in the form of heat that is produced as a result of physical activity, energy used for metabolic processes such as digestion and absorption of nutrients, synthesis of body tissues, and energy lost when the animal is in a resting state (Just, 1982). This heat is transferred to the enviroment via radiation, convection, conduction, and evaporation (Blaxter, 1989), and is known as total heat production (**THP**).

Total heat production is composed by two forms of heat: heat increment (**HI**) and fasting heat production (**FHP**). Fasting heat production is comparable to NE for maintenance (**NE_m**; van Milgen et al., 1998) because it is the energy that is used by the animal in a basal metabolic state, for voluntary activity and thermoregulation (Just, 1982). Therefore, THP is the sum of the energy expenditure by the resting animal plus the heat that is produced while the animal metabolizes nutrients and synthesizes muscle and adipose tissue for growth or reproduction processes (van Milgen and Noblet, 2003). Net energy is then the sum of HI and the energy retained (**ER**) in the body in the form of protein and lipids, deposited in products such as milk, or used for the growth of fetuses (Noblet, 2013). By correcting ME for HI and FHP, it is possible to calculate NE (Li et al., 2018) according to the following equations [3], [4]:

$$NE = [(ME - HI)] \quad [3]$$

$$NE = [(ER + FHP)] \quad [4]$$

Estimation of NE_m requires knowledge about the physiological pathways that are related to regular animal development, its basal activity and growth rate, the reaction rates, and the different fates that energy has on those activities. However, the complexity of estimating these processes results in estimates that are not always accurate (van Milgen and Noblet, 2003). Thus, FHP may be a better value to be used to estimate NE_m.

Energy is often measured as *Joule* (J), which is the international unit for heat or work, but, *the calorie* (cal), the metric heat unit, is also accepted for reporting of energy because this unit is equivalent to 4.184 J (Kleiber, 1972). Calculation of THP is possible if consumption of O_2 and synthesis of CO_2, CH_4, and urinary N is known (Brouwer, 1965), [5]:

$$\text{THP, kcal} = [(3.866 \times O_2 + 1.200 \times CO_2 - 0.518 \times CH_4 - 1.431 \times \text{urinary nitrogen})] \quad [5]$$

Net energy of a growing animal, therfore, is composed of the energy that is retained in the animal plus the energy that is spent when these processes are performed, thus, a more general equation may be used to determine NE from THP, FHP and ME (Noblet et al., 1993b), [6]:

$$NE = (ME - THP + FHP) \quad [6]$$

CALORIMETRY PRINCIPLES

Total energy outflow from animals consists of first, the heat that is produced from digestion, metabolism, and storage of nutrients in the body. Second, the heat transferred from the body to the environment during physical activity, and third, the basal metabolic rate (**BMR**), which is the amount of energy used to maintain vital activities while the animal is resting and to maintain body temperature (Levine, 2005). The percentage that each of these energy losses contribute to THP vary across species and is highly related to the growth and development of the animal as well as its behavior and the environment (van Milgen et al., 1998).

Direct Calorimetry

Direct calorimetry measures the heat that is transferred from an animal to the environment (Benzinger and Kitzinger, 1949), which represents the energy that the animal irradiates. Three systems were developed using direct calorimetry. The isothermal system, consists of an insulated coat or bilayer wall that measures the difference in temperature between the outer surface and the inner surface that is affected by the heat released from an animal located inside (Jequier et al., 1987). The adiabatic system uses a refrigerant liquid to transport the heat produced by the animal to an analyzer, and gain or loss of temperature is avoided by isolating the liquid (Webb et al., 1972). The convection system uses air as the heat transporter and works like the adiabatic system with the difference that the airflow acts as the heat driver

(Snellen, 2000). The heat produced by the body inside the chamber is collected by the air stream and is then analyzed by difference taking into account the heat capacity of the air (Levine, 2005).

Indirect Calorimetry

Indirect calorimetry estimates THP from the gas exchange produced by the animal in the respiration process. In this method, the amount of O_2 consumption and the CO_2 produced are used in a prediction equation to deliver an estimation of heat production (Brouwer, 1965). Gas exchange may be measured by collecting the total amount of air expelled from an animal in a container and the composition of this is analyzed to determine the concentration of the gases, and an oxygen-containing tank is used to measure the oxygen disappearance rate (Matarese, 1997). Another example and one of the most commonly used approaches in indirect calorimetry is called an open-circuit system (**OCS**). This system requires analysis of the composition of the ingoing and outgoing air in a calorimeter chamber to determine the amount of air that is taken in by the animal (Miller and Koes, 1988). The concentration of O_2 consumed and CO_2 and CH_4 produced by the animal is analyzed to determine disappearance of O_2 and total production of CO_2 by difference. Another system is called the confinement system. This procedure places an animal in a completely sealed chamber, and the concentration of O_2,CO_2 and CH_4 is analyzed in a short period of time (e.g.15 minutes) to calculate the rate of concentration changes (Lachica et al., 1995). The volume of the chamber is measured to calculate the total amount of air that is contained in the chamber (Blaxter et al., 1972). The last example of the indirect calorimetry system is the closed- system. This method uses animals placed in an air-tight chamber with a known volume, and CO_2 and water vapor that is produced are removed from the air and the O_2 is returned to the chamber (Miller et al., 1981). The confinement and closed-circuit systems should be used only for a short time to avoid animal suffocation.

ENERGY RETENTION AND HEAT PRODUCTION

Energy retention (**ER**) is determined to calculate the efficiency of the animal when fed a specific diet. Energy is stored in the body as protein and fat when the animal is growing, fattening, gestating or milking (Verstegen et al., 1973). The efficiency is determined based on energy intake, which is influenced by type of feeding (restricted or *ad libitum*), genotype, health status, and age of the animal (Noblet and van Milgen, 2004), and the energy that is stored in the body. The most commonly used techniques to determine ER include the comparative slaughter technique, the carbon-nitrogen balance technique, and determination of THP to be subtracted from the ME value. However, the use of the dual-energy x-ray absorptiometry (**DXA**) technique may also be used to measure body composition which can be associated with the energy that is contained in muscular and adipose tissue (Mitchell and Scholz, 2008).

Comparative Slaughter Technique

In this technique, an animal is sacrificed at the end of an experiment to compare its body composition to the one of an animal with similar characteristics that was slaughtered at the beginning of the experiment (Le Dividich et al., 1994). This technique allows for determination of the energy that was used to synthesize muscle and adipose tissue by differences in the weight of those tissues between the 2 slaughtered animals (Quiniou et al., 1995). However, due to variation in body deposition of protein and fat among animals, even when they belong to the same litter, it is always possible that the values obtained from that comparison are biased, which usually results in lower calculated values for NE than the ones obtained when using other techniques (Velayudhan et al., 2015).

Carbon-Nitrogen Balance Technique

The carbon-nitrogen balance technique is used to determine the energy balance in studies where the concentration of those 2 elements is analyzed in diets, feces, and urine to account for the intake and output of energy (Müller et al., 1999). Therefore, the difference between input and output of carbon and nitrogen is considered the value of the ER because energy is retained in the form of protein and lipids in the body (Le Bellego et al., 2001). In this technique, energy in the form of protein is calculated based on the nitrogen difference, and the amount of carbon that is retained in the form of protein is estimated using a fixed number (76.08%; Blaxter, 1989). The carbon contained in the form of fat is subsequently calculated from the difference between the carbon balance and the carbon retained in protein to calculate the energy retained as lipids (Noblet et al., 1987).

Fasting Heat Production

As previously described, FHP is the thermic energy utilized to maintain vital activities in the body using the energy reserves that have been accumulated in the fed state. Therefore, it can be inferred that in a fasting state, the animals reduce protein synthesis and accretion and limit lipid synthesis for energy storage, to start mobilizing those energy reserves and supply the energy required for maintenance. Thus, FHP provides a reference for the energy that needs to be subtracted from the ME value to estimate the actual value of energy that is used for productive purposes (Holmes and Breirem, 1974).

Fasting heat production is usually calculated using the equation proposed by Brouwer (1965), [5], but prediction equations have also been developed to estimate FHP based on animal body weight (Brown-Brandl et al., 2004). Those equations, however, vary due to multiple factors such as breed, age, weight, etc. (Tess et al., 1984), as well as the quality of the environmental

conditions where animals are kept, because animals with poor health status tend to have greater FHP (up to 8% more; Meer et al., 2019).

Dual-Energy X-ray Absorptiometry

Alternative procedures to measure body composition during different production phases were developed because of the impossibility of the comparative slaughter technic to be performed twice in the same animal (Blaxter, 1989). Therefore, the dual-energy X-ray absorptiometry (**DEXA**) may be used to measure the chemical composition of a live animal in terms of protein, fat, and bone composition (Svendsen et al., 1993). This technique uses two X-ray lights to measure the muscle, adipose, and bone tissue by differences in the intensity of the X-ray light that goes through the body, and such mitigation of the intensity is then contrasted with standard values to determine body composition (Kipper et al., 2019). The accuracy of the DEXA procedure has been tested in pigs and humans (Svendsen et al., 1993; Mitchell and Scholz, 2008) demonstrating that DEXA may be a reliable procedure to measure body composition. However, the procedure has not been validated and at this time, the DEXA procedure has not been used to generate NE values in diets fed to pigs and no NE systems have been developed based on this procedures.

NET ENERGY SYSTEMS

The French System

The French NE system was proposed in the early 1990s, with the purpose of generating values for NE that included new techniques for chemical analysis of diets and advanced knowledge about the dietary contribution of energy, especially from carbohydrates (Noblet et al., 1994a). This system proposed that a large proportion of the ME is used for maintenance, and

only the ME available after the requirements of the animal are met, can be used for production purposes (Noblet et al., 1993b).

The French system was designed to calculate NE_m by feeding animals with a wide variety of diets and to evaluate the effect of diet composition on animal HP by the use of indirect calorimetry. The efficiency of the use of NE was then determined, generating energy expenditures by pigs in different growth stages (Noblet et al., 1993b). This efficiency was believed to be dependent on environmental, productive, and genetic factors (e.g., temperature, feeding frequency, breed, and body weight); however, it was demonstrated that the NE efficiency can be determined using the indirect calorimetry approach (Noblet et al., 1994b).

The Dutch System

The Dutch system was developed in the Netherlands by the Central Bureau for Livestock Feeding (Stewart, 2007), where analyses of the feed used for animal nutrition are performed to determine both chemical composition and nutrient digestibility in these materials. Having the concentration and digestibility of nutrients, the energy value of the feed can be determined (CVB, 2016). The procedure that is used in this system consists of a series of metabolism experiments where the input and output materials are collected, and the nutrient composition is analyzed. Therefore, the difference between intake and output determines the portion of the energy that has been absorbed or retained by the animal, providing digestibility coefficients that are applied for protein, fat, digestible carbohydrates, and fiber (CVB, 2016).

In the Dutch system, energy losses in the form of heat are not measured; however, the analysis of the carbohydrate component is more specific than in the other systems. Thus, non-starch polysaccharides, fermentative starch, digestible starch, and digestible sugars are separated to determine coefficients of digestibility of each of these components separately (CVB, 2016).

The Danish System

The Danish system is also known as the potential physiological energy (**PPE**) system (Kil et al., 2013). The PPE system was presented for the first time by Boisen and Verstegen in 1998, as an alternative procedure because, in the other NE systems, the value for NE is obtained when the animals are under specific environmental and physiological conditions (Velayudhan et al., 2015). Therefore, a more comprehensive system was developed, where not only the digestibility of nutrients is taken into account but also the theoretical yield of ATP during the oxidation of feed components (Szabó and Halas, 2013).

Thus, the PPE system ignores the characteristics of the animal in terms of breed, age, or environmental conditions that may influence the efficiency of nutrient oxidation (Kil et al., 2013). However, the system applies digestibility values that have been obtained in experiments *in vitro* to obtain the greatest percentage for the oxidation of nutrients. Therefore, the PPE system can be applied to any animal because the values obtained with this technique for individual ingredients are additive in mixed diets (Boisen, 2007).

Prediction Equations

Prediction equations have been developed to estimate energy values in feed ingredients and diets based on their nutritional composition. These equations attempt to deliver an accurate energy value using coefficients that were obtained from different feed ingredients after analyzing chemical composition and digestibility values (French and Dutch systems), or the potential for ATP generation (Danish system, Noblet et al., 1993a). The objective of the prediction equations is to calculate NE values in feed ingredients, which may be used in diet formulation. By using this system, the natural variation in chemical composition in feed ingredients is avoided and the effects of dietary treatments on growth performance may be predicted (Fairbairn et al., 1999).

The French system developed 11 prediction equations based on proximate analysis of starch (**ST**), ether extract (**EE**), crude protein (**CP**), crude fiber (**CF**), and hemicellulose (**Hemi**), which is determined by the difference between neutral detergent fiber (**NDF**) and acid detergent fiber (**ADF**; Noblet et al., 1994a), as well as the digestible values of each of these nutrients (**dST**, **dEE**, **dCP**, **dCF**, and **dHemi**, respectively). The difference between digestible organic matter and the sum of digestible chemical components (**DRes1**, **2**, or **3**) are also included in these equations.

The use of DRes1, DRes2 and DRes3 values depends on the digestibility coefficients that are available to calculate NE. For example, DRes1 is calculated when values of sugar (**SU)** and Dhemi has been determined, therefore those values can also be substracted from the digestible organic matter. Likewise, DRes2 is used when only values of dCP, dEE, ST, and dADF have been determined, and DRes3 is applied if values for digestible crude fiber (**dCF**) were calculated instead of dADF values (Noblet et al., 1994a).

The prediction equations are presented below (Noblet et al., 1994a), [7-17]:

$$NE\ (MJ/kg) = 2.73 \times DCP + 8.37 \times DEE + 3.44 \times ST + 0 \times DADF + 2.93 \times DRes2 \quad [7]$$

$$NE\ (MJ/kg) = 2.69 \times DCP + 8.36 \times DEE + 3.44 \times ST + 0 \times DCF + 2.89 \times DRes3 \quad [8]$$

$$NE = 0.843 \times DE - 463 \quad [9]$$

$$NE\ (MJ/kg) = 0.703 \times DE + 1.58 \times EE + 0.47 \times ST - 0.97 \times CP - 0.98 \times CF \quad [10]$$

$$NE\ (MJ/kg) = 0.700 \times DE + 1.61 \times EE + 0.48 \times ST - 0.91 \times CP - 0.87 \times ADF \quad [11]$$

$$NE = 0.870 \times ME - 442 \quad [12]$$

$$NE\ (MJ/kg) = 0.730 \times ME + 1.31 \times EE + 0.37 \times ST - 0.67 \times CP - 0.97 \times CF \quad [13]$$

$$NE\ (MJ/kg) = 0.726 \times ME + 1.33 \times EE + 0.39 \times ST - 0.62 \times CP - 0.83 \times ADF \quad [14]$$

$$NE\ (MJ/kg) = 2{,}796 + 4.15 \times EE + 0.81 \times ST - 7.07 \times Ash - 5.38 \times CF \quad [15]$$

$$NE\ (MJ/kg) = 2{,}790 + 4.12 \times EE + 0.81 \times ST - 6.65 \times Ash - 4.72 \times ADF \quad [16]$$

$$NE\ (MJ/kg) = 2{,}875 + 4.38 \times EE + 0.67 \times ST - 5.50 \times Ash - 2.01 \times (NDF - ADF) - 4.02 \times ADF \quad [17]$$

The Dutch system recently developed a new prediction equation for NE that takes into account digestibility values of nutrients that were obtained using indirect calorimetry (CVB, 2016). By the use of the different data-bases of chemical composition and digestibility values of a considerable number of feed ingredients, the novel equation was established and is presented below (Blok et al., 2015), [18]:

$$NE\ (MJ/kg) = (11.70 \times dCP + 35.74 \times dCFat_h + 14.14 \times (Starch_{AM} + 0.9 \times Sugars_e) + 9.74 \times (fNSP + CF\_Di \times Sugars_f) + 10.61 \times AA + 19.52 \times BA + 14.62 \times PA + 12.02 \times LA + 20.75 \times Eth + 13.83 \times Glycerol) / 1000 \quad [18]$$

The variables that are included in the novel equation are values found in databases for digestible crude protein (**dCP),** digestible crude fat after acid hydrolysis (**$dCFat_h$**), starch analyzed according to the amyloglucosidase method (**$Starch_{AM}$**), fermentable non-starch polysaccharides fraction (**fNSP**), a correction factor for sugars (**CF_Di**), sugars that are fermented by bacteria in the hindgut (**$Sugars_f$**), acetic acid (**AA**), butyric acid (**BA**), propionic acid (**PA**), lactic acid (**LA**), ethanol (**Eth**), and glycerol (Blok et al., 2015).

As was previously described, the Danish system argues that it is possible to determine the NE content of a feedstuff disregarding the physiological characteristics of the animal. For this aim the PPE system developed coefficients to be applied to the different nutrient digestibilities and the PPE values are then determined (Kil, 2008). The equation needed to calculate the PPE value of a feedstuff is presented below (Boisen, 2007), [19]:

$$PPE\ (KJ/g\ DM) = 9.9 \times RDCP + 31.7 \times RDCF + 11.7 \times EDC + 7.0 \times FERMC - 2.8 \times EIDMi\ /7{,}375 \quad [19]$$

The values that are included in the PPE equations are: the *in vitro* ileal degestible CP content (**RDCP**), the ileal digestible crude fat content (**RDCF**), the *in vitro* ileal digestible carbohydrates (**EDC**), fermentable carbohydrate (**FERMC**), and the enzyme undigested ileal DM (**EIDMi**).

CONCLUSIONS

Digestible energy and ME are valid methods to determine energy expenditure in animals; however, NE may be more accurate because the energy in form of heat is also taken into account as energy loss. Indirect and direct calorimetry principles are well known methods used to determine HP; however, the indirect calorimetry approach is more commonly used because of its practicality and the lower cost of implementation.

There are a number of techniques to determine ER in animals as well as HI from metabolism of nutrients, but advantages and disadvantages need to be considered to choose the most appropiate technique. Some NE systems have been developed using different elements to obtain prediction equations that can be used to predict the response of the animals when a specific ingredient or a complete diet is being used.

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CHAPTER 3: DESCRIPTION OF THE SWINE CALORIMETER UNIT STRUCTURE

ABSTRACT

The digestible energy (**DE**) and the metabolizable energy (**ME**) systems are the most commonly used energy systems in the U.S., but the net energy (**NE**) system may be more accurate due to inclusion of total energy losses in this system. The indirect calorimetry procedure is often used to determine gas exchanges, which are needed to calculate NE values of diets. The swine calorimeter unit (**SCU**) has been constructed at the University of Illinois at Urbana-Champaign. The objective of the SCU is to determine NE of diets and ingredients fed on an ad-libitum basis to group-housed pigs in all phases of production. The SCU allows for calculating NE based on the indirect calorimetry procedure. There are 6 calorimetry chambers in the SCU. Each chamber is airtight and has a capacity to hold 4 to 10 growing-finishing pigs depending on size. The SCU allows for collecting feces and urine to calculate DE and ME, and each chamber is equipped with a fresh air supply system. A regulator unit controls humidity and temperature in each chamber, and a gas analyzer system is used to measure the gas exchange in the chambers. Those systems are placed in equipment rooms next to the chambers. The concentration of oxygen (**O_2**), carbon dioxide (**CO_2**), and methane (**NH_4**) in the air is measured every 10 min and these measurements are used to calculate total heat production from each chamber. The SCU also contains an air conditioned feed storage room to maintain the experimental diets under controlled conditions. A master computer room, where animals and equipment are monitored, and a mechanical room where facility entrances are located, are also included in the unit.

Keywords: calorimeter, group housing, net energy, pigs.

INTRODUCTION

The most commonly used energy system in the U.S. is a system based on metabolizable energy (**ME**), although the digestible energy (**DE**) system is also used. Values for DE and ME are calculated after subtraction of the energy that is excreted in feces and urine, respectively. Theoretically, ME also takes into account the energy that is lost in combustion gases, but in most cases, these gases are ignored in calculations of ME values for pigs. However, it is believed that the most accurate system to determine energy requirements is the net energy (**NE**) system, because, in addition to energy lost in feces and urine and gas, the NE system also accounts for the energy lost in the form of heat (Noblet and van Milgen, 2004).

Different procedures have been developed to calculate NE of diets and feed ingredients used in animal feeding (Noblet, 2013; Velayudhan et al., 2015b). Among these methods, the indirect calorimetry procedure is most often used because NE can be calculated based on the consumption of oxygen (**O_2**) and the production of carbon dioxide (**CO_2**) and methane (**CH_4**). Although prediction equations to estimate NE of feed ingredients and diets have been published (Noblet, 2000; NRC, 2012; CVB, 2016) it is possible that more accurate prediction equations can be developed if newer analytical techniques and more accurate digestibility values are used, compared with what was used to develop previous prediction equations. Values for NE can be quantified from the energy ingested from feed and the energy lost after the digestion process. Therefore, it is important that accurate values for characterization of the ingredients as well as corrected values for digestibility of nutrients are used. Likewise, results of research conducted during recent decades have demonstrated that use of different feed technologies may influence energy values of diets fed to pigs. Thus, particle size, pelleting, extrusion, enzyme addition, and

use of other additives may affect the amount of energy pigs obtain from diets, but none of these technologies are incorporated in current NE systems.

The chemical composition of diets and digestion of nutrients need to be combined with measurements of gas exchanges to calculate NE values. To determine gas exchange using indirect calorimetry, respiration chambers are used. Different types of respiration units have been designed to accommodate different species (Benedict and Homans, 1912; Pinares-Patiño and Waghorn, 2012; Maia et al., 2015). In most cases, calorimeter chambers allow for only one or two animals at the same time being placed in the chamber (Benedict and Homans, 1912; van Milgen et al., 1997; Velayudhan et al., 2015a). However, pigs are social animals and under commercial conditions, pigs are kept in groups, and it is likely that group-housed pigs have different energy expenditures than individually housed pigs, which may affect estimated values for NE. It is possible, therefore that NE values that accurately reflect what is obtained in commercially housed pigs, need to be determined in group-housed pigs. Likewise, because commercial pigs usually are allowed *ad libitum* access to feed, and because the level of feed intake may affect digestibility of nutrients and energy and post-absorptive energy metabolism, it is possible that NE values that are obtained in pigs allowed *ad libitum* intake of feed are more representative of commercial pigs than if pigs are restricted in their feed intake. Therefore, the Swine calorimeter Unit (**SCU**) has been constructed to determine NE values in feed ingredients and diets consumed by group-housed pigs that are allowed *ad libitum* access to diets.

UNIT MEASUREMENTS AND EQUIPMENT

The Swine Calorimeter Unit is located at the Swine Research Center (**SRC**) at the University of Illinois at Urbana-Champaign. The outside of the SCU measures 28.04 × 5.11 m.

The unit is a wood framed construction that is placed on a steel chassis, with oriented strand board walls, a wood truss roof, and a plywood floor. All surfaces on the inside are coated with sprayed-on plastic for water-tightness. Inside, the unit consists of a feed storage room, an access corridor, 3 equipment rooms, 6 calorimetry chambers, a computer room, and a mechanical room (Figure 3.1).

All rooms are connected by the access corridor, which has a length of 20.55 m (Figure 3.2). The access door for personnel and animal entrance is located on the west wall of the building, the size of this door is 2.01 × 1.06 m. Inside, the animals are guided to the calorimeter chambers using a wooden ramp. Three water faucets and four-fixed connections to the power washer lines are located in the access corridor. Electricity outlets are located throughout the corridor.

The feed storage room (Figure 3.3) is where experimental diets are kept under temperature controlled conditions to avoid spoilage. Room temperature in the feed storage room is maintained at 15 ± 2 °C, using a Bard® T30S1D control unit (BARD HVAC, Bryan, OH). In addition to controlling temperature, this equipment also has a dehumidification circuit to control air humidity in the room. The feed storage room has a volume of 44.3 m^3. The inside dimensions are 3.43 × 4.84 m and the height is 2.67 m. There is a door (1.99 × 1.84 m) to the outside, which allows entrance of feed stored on pallets or in feed bins. There is also a narrower door (2.01 × 1.06 m) from the feed room to the corridor in the SCU for movement of feed from the feed room to the chambers using a hydraulic lifted cart (JRMC-11ELT, Lift Products INC, Waukesha, WI).

All equipment in the SCU is connected to the master computer, which is located in the computer room (Figure 3.4). The master computer monitors the operational quality of all

equipment in the SCU and also monitors the well-being of the animals. The volume of this room is 29.84 m^3, with inside measures of 3.02 × 3.70 × 2.67 m.

The mechanical room is where utility entrances are located. The electrical service entrance box, a water backflow prevention system, a fresh air supply fan, and a stationary power washer are also located in this room. The volume is 8.30 m^3; with inside dimensions of 3.02 ×1.03 × 2.67 m. (Figure 3.5).

CHAMBER STRUCTURE

Six calorimetry chambers are located in the SCU. Each chamber is composed of a main section for animals and a secondary section to collect feces and urine (Figure 3.6). The main section has a volume of 6.5 m^3. The inside dimensions are 1.83 × 1.97 m, and the height is 1.8 m. The door of the main chamber is air-tight, has a gasketed surface, is side-hinged, and contains 3 rubber-metal handles for closing. The secondary section has a volume of 3.1 m^3. The inside dimensions are 1.83 × 1.97 m, and the height is 0.86 m (Figure 3.7). The door of the secondary chamber is air-tight by means of a gasketed surface, and has 8 rubber-metal handles for complete closing; this door can be removed to allow fecal and urine collection. The ceiling and walls in the main chambers are constructed from a wood-coated frame with sprayed-on plastic and the floors are galvanized steel modular slotted flooring with the animal contact surface made of a series of spaced triangular bars. The floor module is self-supporting at the ends by means of an engineered steel under-slat truss system. The contact surface is deformed to improve hoof traction. The support truss is designed for the maximum animal load and module length (Table 3.1). An air supply duct and diffuser is located in the ceiling of each chamber, and an air-outlet is located in the side of each chamber to provide the air exchange needed. The chamber contains a stainless

steel wet-dry feeder with a capacity of 30 kg (Thorp Equipment Inc., Thorp, WI). An auxiliary drinker is available in each chamber to ensure free access to water.

The secondary chamber has 4 flat stainless steel wire mesh screens with openings of 1,190 microns for feces collection (Figure 3.8); measurements of these screens are 0.91 × 1.97 m. The screens are placed in parallel and in two rows with a 10 cm separation between screens to avoid sample loss during collection. Feces are collected every day within the 1-hour opening period. During this time, both the main and secondary sections of the chambers are opened. To remove the feces that are present on the screen floor, the animal allocation section is opened, the screens in the upper row in the secondary section are pulled out from their mounting, and the feces on the screen are collected. After cleaning the upper screens and placing them back under the slatted floor, the lower screen row is pulled out to collect the remaining fecal material that may have been voided while the upper screen row was pulled out for collection of feces. The 2 urine pans are placed below the screens and have a total capacity of 100 L. The pans are equipped with a manual valve, which allows for collection of urine from the access corridor. During collection days, valves are opened once per day to collect the urine in plastic buckets. Once the pans are empty, the valve is closed and urine will be captured in the pans. To avoid nitrogen loss from the urine, 125 ml of 6*N* HCl are placed in the urine pans every day.

EQUIPMENT ROOMS

All air handling equipment for the chambers is located in the 3 equipment rooms (Figure 3.9). The equipment includes systems to control temperature and relative humidity inside the chambers, a system for the fresh air supply to the chambers, and systems to analyze air samples

for oxygen (**O_2**), methane (**CH_4**), and carbon dioxide (**CO_2**). Each equipment room has a volume of 27.64 m^3, with inside dimensions of 2.95 × 3.51 m × 2.67 m.

Temperature and Humidity Control System

The temperature and humidity in the chambers are maintained by 6 Parameter Generation and Control (**PGC**) units (Model 9241-2220-B1D0000; Parameter, Black Mountain, NC), which are located in the 3 equipment rooms, with 2 PGC units per room. The PGC units control the temperature with an accuracy of ± 0.1°C and relative humidity is maintained with an accuracy of ± 0.5%. This level of precision is ensured by the use of the *dew point control system*, which operates by manipulating the air temperature going through the PGC unit and the temperature of the water spray that saturates the air with moisture and thus, controls the humidity and temperature in the chamber (Dyer, 2012). Because temperature and humidity in each chamber is individually controlled by one PGC unit, different temperature and humidity conditions can be maintained in each chamber.

The air blower in each PGC unit has a capacity of 700 to 1,100 m^3 of air per hour. The unit is a 316 grade stainless steel construction, with electricity requirements of 208/230V, 3-Phase, 60Hz, 22.5 full-load amperes, and 14.1 rated load amperes. The weight of each PGC unit is 522 kg and dimensions are 0.81 × 1.02 × 1.63 m. The rated maximum heat of rejection for each unit is approximately 7,300 watts/h. The PGC unit provides instant readings of temperature and humidity in the chamber (Figure 3.10), and allow programming for automatic cycles, set points, and tuning parameters, and has a RS-232 connection to the master computer for continuous monitoring. The equipment also contains an alarm system that is activated if the temperature or the humidity in the chamber deviates from the set allowances.

Fresh Air Intake System/ Air Exchange

Two fresh air supply systems are placed in each equipment room. These systems provide clean air for the air exchange into the chambers and provide the baseline needed for the gas analyzers (Figures 3.11). The fresh air intake system/ air exchange consist of a centrifugal inline fan, which has a maximum rated airflow of 293 m^3/h, and its housing is constructed of galvanized sheet metal (Fantech, Lenexa, KS). The AccuValve® (Accutrol llc, Monroe, CT) is also part of the system; it has a length of 56 cm and a diameter of 15 cm. The AccuValve® divides the airflow in 2 equal flows, which pass through the airflow sensor; a measure of this airflow is sent to the digital controller where the airflow set point is calibrated. The controller modulates the blades inside the AccuValve® to achieve the airflow determined by the set point and moderates the airflow to be sucked into the calorimeter chamber by the fan. The AccuValve® has an accuracy of +/- 5% and a maximum airflow rate of 509 m^3/h and is equipped with 90 cm of a 15 cm diameter PVC pipe on each side to allow maximum accuracy according to manufacturer recommendations.

The air exchange in the chamber is set by the AccuValve® and chamber pressure is regulated by a manual rotary plate valve located in the exhaust duct, which allows chamber air to vent to the outside of the building. The manual valve is set to maintain a small positive pressure in the chambers, and to avoid entrance of external air to the chamber. A manometer (Dwyer ®, Michigan City, IN) with an accuracy of 3% is attached to the exhaust pipe, to maintain the chamber pressure at 174.19 Pascal at all times.

Gas Analyzers

There are 3 gas analyzer systems in the SCU with one system located in each equipment room (Figure 3.12). Each system (*Classic Line*, Sable System International, North Las Vegas,

NV) analyzes air from 2 calorimeter chambers, and consists of 2 pumps, a multiplexer, a sub-sampler, a humidity sensor, an O_2 analyzer, a CO_2 analyzer, and a CH_4 analyzer. The gas analyzer system provides readings of O_2, CO_2, and CH_4 from a subsample of air collected from the chamber return duct. The pumps funnel the air from the return duct to the multiplexer, as another pump collects a sample from the fresh air supply duct to the multiplexer. The multiplexer is programed to select one of the 3 lines of airflow every determined period to be sent to the sub-sampler. The sub-sampler pulls 250 ml ± 10% per minute of air from the chamber through the gas analyzers. Before the air stream enters the gas analyzers, it passes through the humidity sensor, which detects water molecules by infrared spectroscopy, generating relative humidity values. The air subsample first enters the CO_2 analyzer, then the CH_4 analyzer, and finally the O_2 analyzer. The gas analyzers provide readings in percentage units. The resolution of the analyzer is 0.0001, but this resolution can vary depending on gas concentration.

Calibration Sequence

The calibration process includes the tune-up of the AccuValve® in the fresh air supply system, the temperature and humidity sensor in the PGC unit, and the standardization of the 3 gas analyzers. Due to the low airflow that is kept in the chambers, an air calibrator is installed to measure the actual air flow modulated by the AccuValve® (Figure 3.13). The calibrator uses an obstruction flow meter based on an American Society of Mechanical Engineers (ASME) standard nozzle (Hy-Grade Metal Products Corp. Syracuse, NY), which is placed downstream from the AccuValve®. A vacuum motor (Ametek Dynamic Fluid Solutions, Whitsett, NC), is used in the calibrator to simulate different pressures and to boost the air stream through the nozzle to maintain the inner pressure range as required, and to reduce the pressure inside the calibrator. To reduce large-scale turbulence, a straightener device is inserted inside the calibrator.

A 120-cm length of 15-cm diameter straight pipe is also installed ahead of the nozzle to obtain reliable pressure readings. A sample of airflow pressure in each side of the nozzle is recorded to measure pressure drop, which is obtained by the use of a digital manometer (Dwyer ®, Michigan City, IN). Those readings are annotated to graph the actual airflow at different AccuValve® settings.

The temperature and humidity sensor (Figure 3.14) is located in the return duct of the chamber that is connected to the PGC unit. This sensor needs to be recalibrated every year. Standardization of the gas analyzers are divided in 2 steps that can be executed separately or in sequence. The first step includes running a pure N flow through the gas analyzers for an hour to remove all air inside the analyzers and the zero concentration of the gases is then determined in each gas analyzer. The second step is to standardize the equipment using spam gasses. In the case of the CO_2 and CH_4 analyzers, the spam gas contains 99% N and 1% CO_2 or CH_4. After 20 minutes of letting the spam gas pass through the analyzers, the spam concentration of the gasses is assessed in the CO_2 and CH_4 analyzers. In the case of the O_2 analyzer, a desiccant containing Dryerite and Ascarite is used to remove excess water and CO_2 in the airflow. The atmospheric O_2 concentration average is 20.944 % (Glueckauf, 1951), and therefore, the concentration range that is set in the analyzer is 20.95 ± 1 %. As a consequence, the gas analyzer can determine small changes in the O_2 concentration.

Dry Cooling System

The dry cooling system (Vertiv Tm, Columbus, OH) is used to provide heat transfer between the PGC unit's refrigeration condensers and the atmosphere outside the building (Figure 3.15). This is achieved because the system contains 2 exhaust fans that exhaust the heat

that is transferred from the PGC's metal surface that contains the refrigerant to the propylene glycol-filled finned tubes (Conradie and Kröger, 1996).

An outside fan-cooled radiator, an electric pump that will distribute the fluid cooler through a manual balancing valve, which produces a pressure difference across the supply and return sides of the glycol loop, and another pump for back up purposes compose the dry cooling system. The fan-cooled radiator dimension is 1.1 × 2.3 × 1.1 m. The radiator is located in an aluminum cabinet outside the SCU. The dry cooler glycol loop includes a 30-L expansion tank. Its dimensions are 0.76 × 0.82 × 0.48 m and it is placed in an aluminum drip-proof case. The dry cooler system can lead 21,370 m^3 of air per hour. The fluid flowrate is 75 L/min, which allows the system to remove 42,202 watts of heat from the system per hour.

Conclusion

The SCU at the University of Illinois contains systems and equipment that control environmental conditions to maintain temperature and humidity in the comfort zone for animals and thereby limit energy losses in form of heat used for thermoregulation. The equipment in the SCU includes an air regulator system that assure an adequate air exchange between the chamber and the atmosphere. The floor space allowance in each chamber is equivalent to what is used in commercial conditions, and the *ad libitum* feeding and drinking supply are intended to maintain normal animal growth. The SCU is also equipped with the instruments needed to detect changes in the concentration of gases, which are used to calculate animal heat production and net energy of diets or feed ingredients.

FIGURES

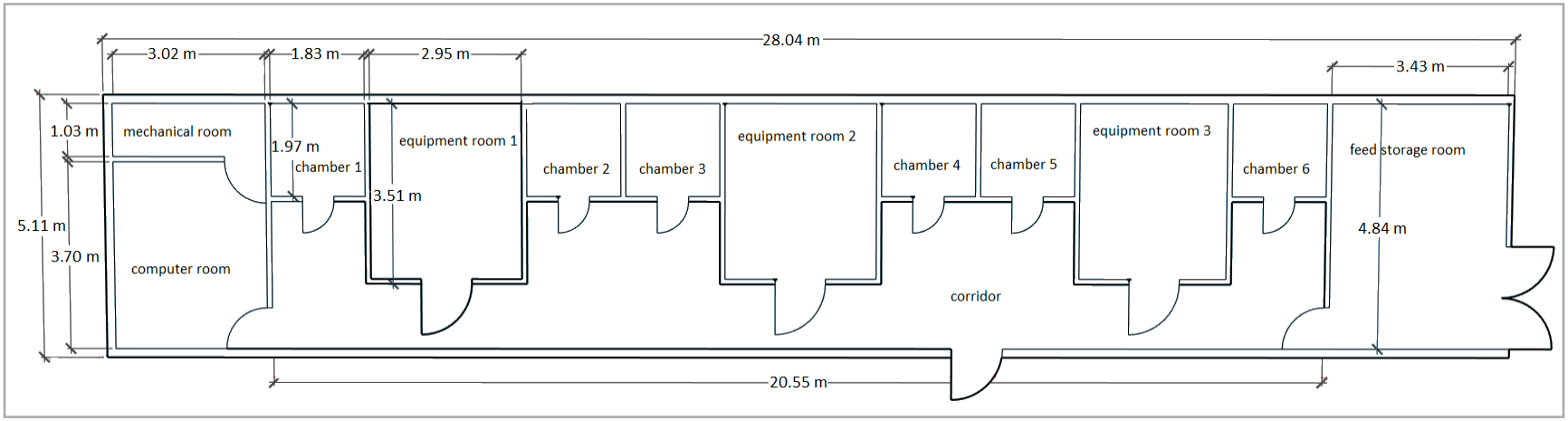

Figure 3.1. Layout of the swine calorimeter unit.

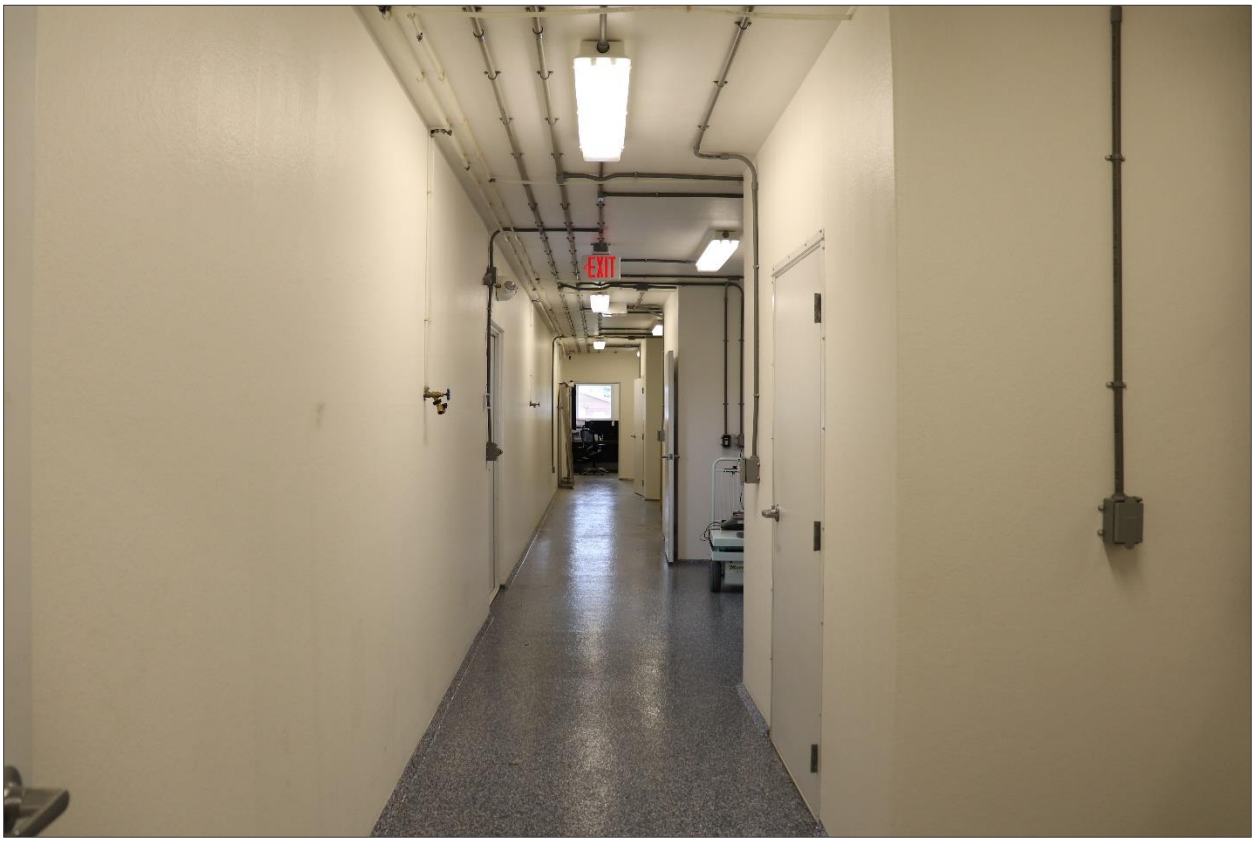

Figure 3.2. Access corridor and access door.

Figure 3.3. Feed storage room.

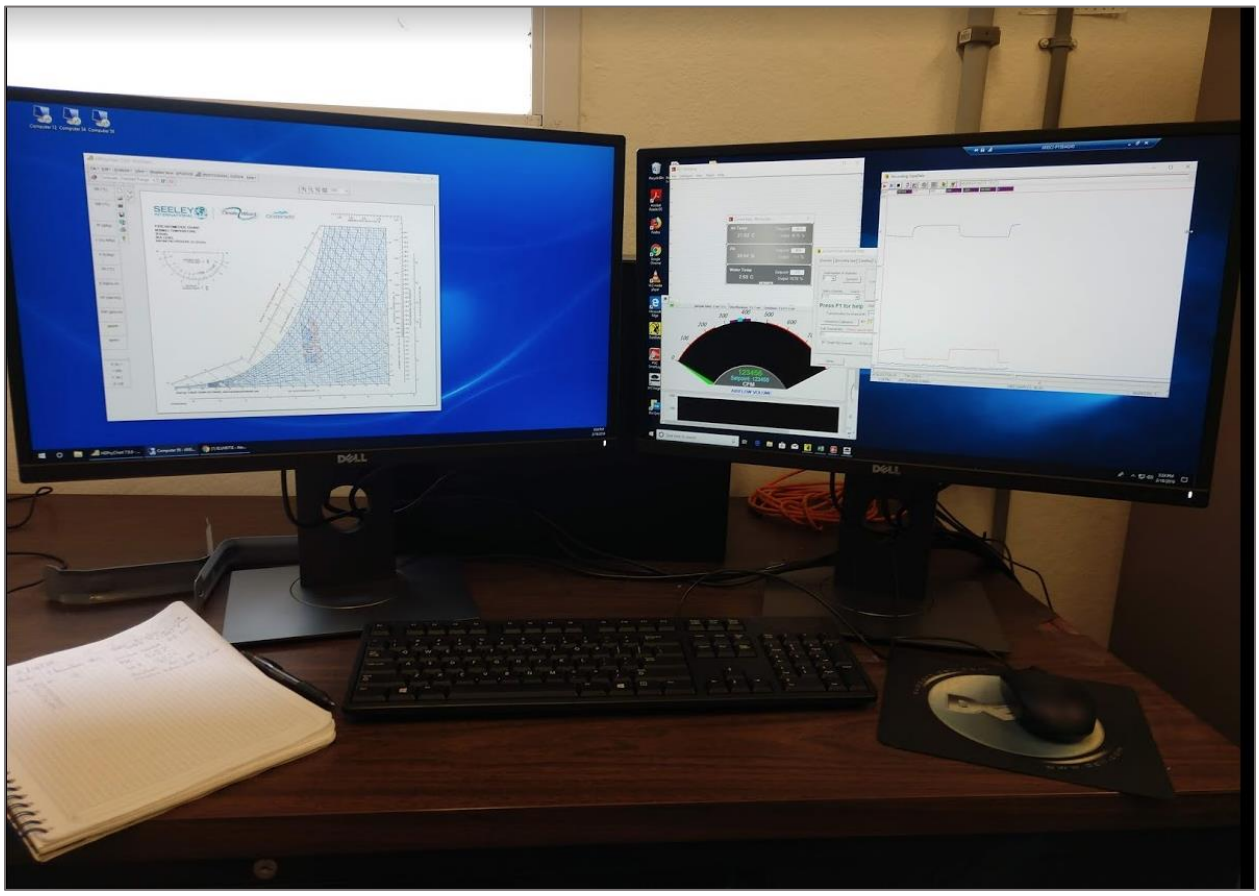

Figure 3.4. Master computer for monitoring.

Figure 3.5. Mechanical room.

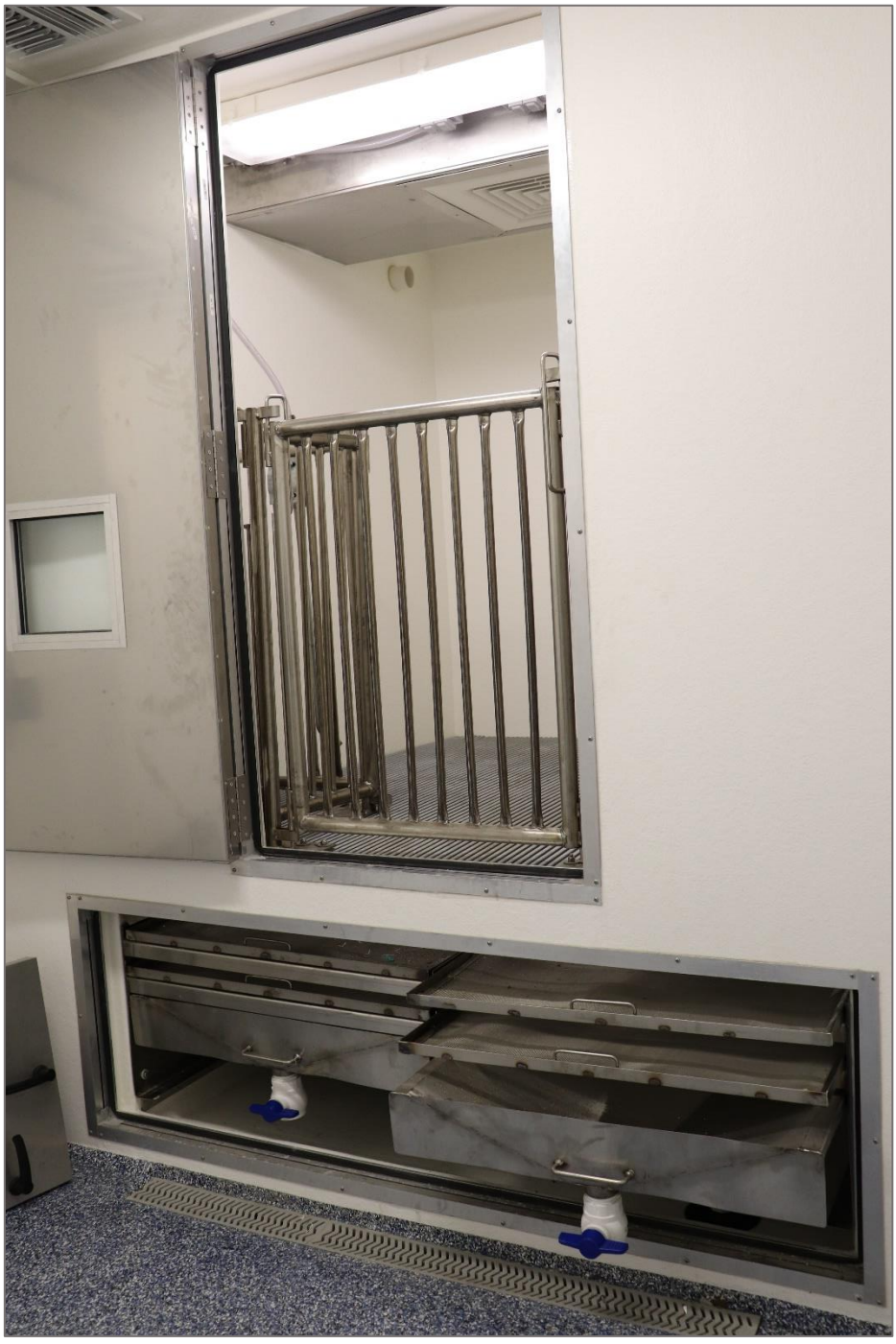

Figure 3.6. Calorimeter chamber.

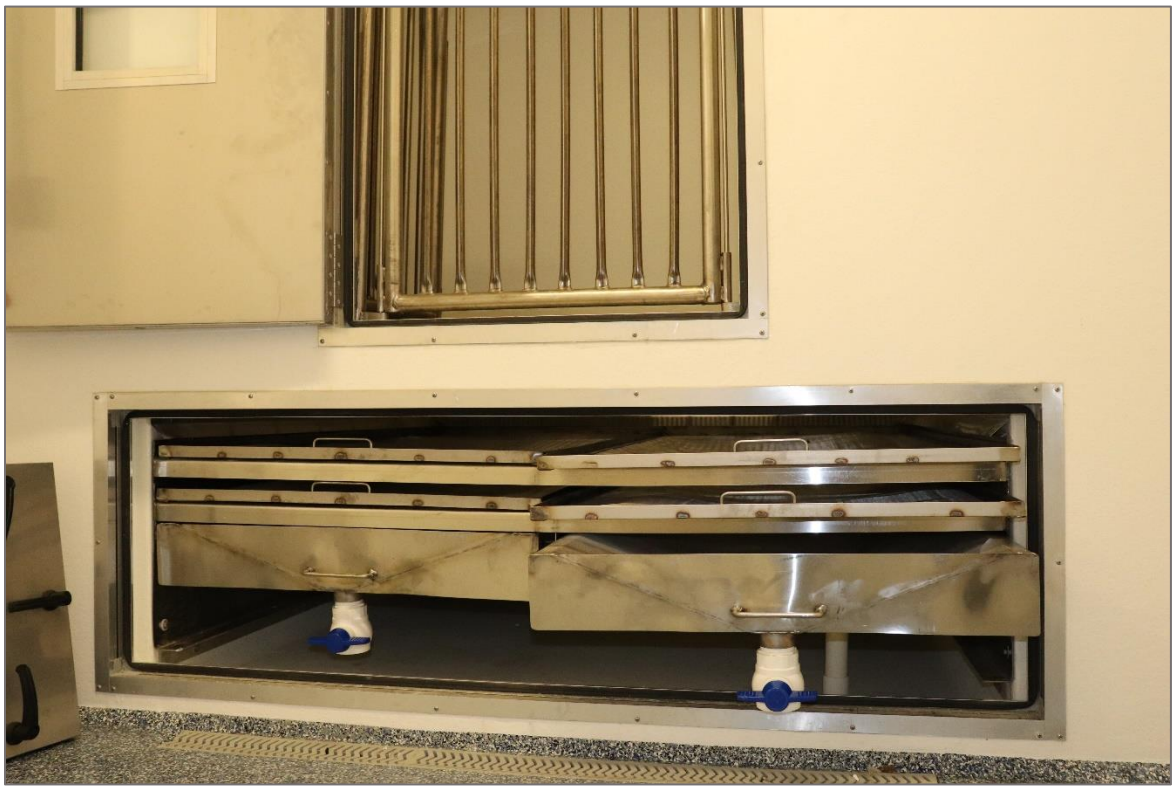

Figure 3.7. Secondary section of the calorimeter chamber.

Figure 3.8. Screens and urine pans inside the secondary section of the calorimeter chamber.

Figure 3.9. Parameter Generation and Control (PGC) unit inside the equipment rooms.

Figure 3.10. Parameter Generation and Control (PGC) unit with display.

Figure 3.11. Fresh air supply system.

Figure 3.12. Gas analyzers system.

Figure 3.13. Fresh air supply system calibrator.

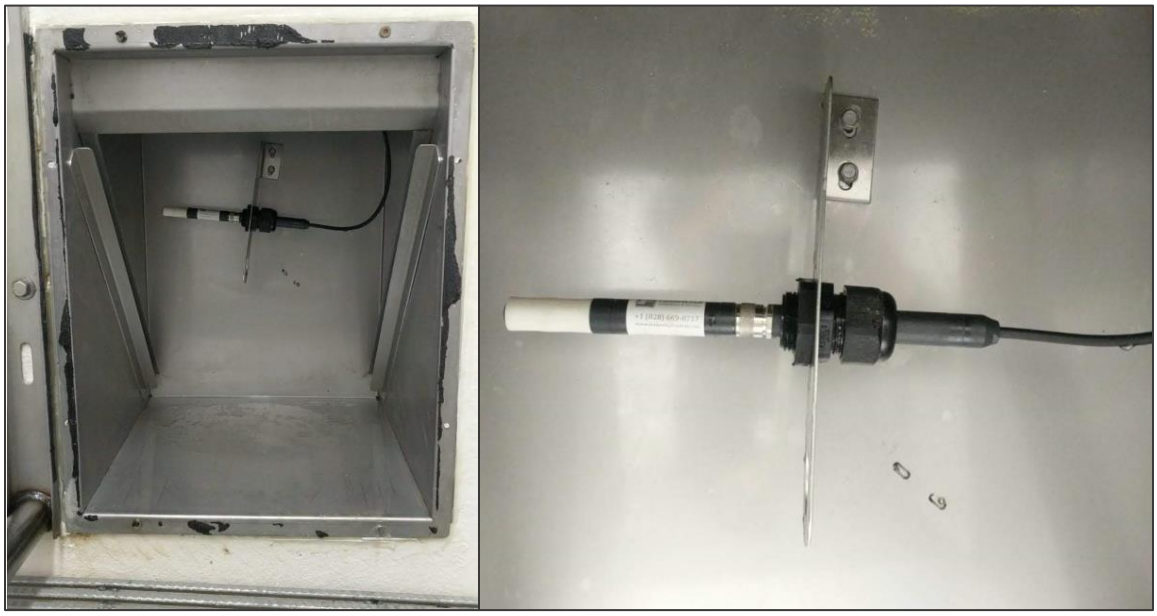

Figure 3.14. Temperature and humidity sensor.

Figure 3.15. Dry cooling system.

TABLE

Table 3.1. Allowance capacity per calorimeter chamber

Description	Number of pigs in chamber
up to 10 kg	Usually not used
11-20 kg	10
21-40 kg	6
41-60 kg	6
61-80 kg	4
81-100 kg	4
100-130 kg	4
Adult pigs in groups	2

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CHAPTER 4: HEAT PRODUCTION AND CONCENTRATION OF NET ENERGY IN THE DIET OF GROUP-HOUSED GROWING PIGS USING A NOVEL SWINE CALORIMETER

ABSTRACT

A 14-d experiment was conducted to test the hypothesis that there are no differences in the estimated values for total heat production (**THP**) and net energy (**NE**) among the 6 chambers in the Swine calorimeter unit (**SCU**) when similar conditions are set and the same diet is fed to 5 pigs per chamber. A common diet based on corn and soybean meal (**SBM**) was formulated to meet or exceed requirements for 25- to 50-kg pigs. Thirty pigs (BW: 38.3 ± 1.8 kg) were randomly allotted to 6 calorimeter chambers with 5 pigs per chamber. Pigs were allowed *ad- libitum* access to the diet for 11 d, and the amount of feed provided was recorded every time the feeder was refilled. On d 12, feed was removed from the feeders and pigs had a 36-h fasting period to determine fasting heat production (**FHP**). The O_2 consumption and CO_2 and CH_4 production, as well as urine N, were measured during the fed and fasting periods to calculate THP. Data were analyzed using the PROC MIXED procedure (SAS Inst. Inc., Cary, NC). Results indicated that there were differences ($P < 0.01$) in THP and RQ among chambers. These differences are likely a result of differences in daily feed intake (CV 6.2%) among chambers, but it is also possible that differences in physical activity contributed to the differences. The CV for DE, ME, and NE among chambers was 1.1, 2.0, and 4.2 %, respectively, and these values were less than the CV for THP, FHP, and heat increment. In conclusion, the NE of a corn-SBM diet was successfully determined in group-housed pigs that were allowed *ad- libitum* access to feed.

Key words: group-housed pigs, heat production, net energy.

INTRODUCTION

Results of recent research have supported the hypothesis that the net energy (**NE**) system is the most accurate system to be used in animal feeding when mixed diets are formulated (Velayudhan et al., 2015). The reason for this observation is that the energy expenditure from animals in the form of heat is ignored in the digestible energy (**DE**) and the metabolizable energy (**ME**) systems (Noblet and van Milgen, 2004), but heat increment is included in calculated values for NE. Values for NE of diets may be calculated using indirect calorimetry (Levine, 2005), but equipment needed for indirect calorimetry is available only at a few research facilities. Therefore, the Swine Calorimeter Unit (**SCU**) was constructed at the Swine Research Center of the University of Illinois at Urbana-Champaign with the objective of determining NE values in ingredients and diets that are provided on an *ad- libitum* basis to group-housed pigs in different production phases. The SCU allows for the calculation of NE values by the indirect calorimetry approach and contains 6 identical chambers. However, before the SCU is fully commissioned, it needs to be demonstrated that the chambers per se are not a source of variation. Therefore, the objective of this research was to test the hypothesis that there are no differences in the estimated values for total heat production (**THP**) and NE among the 6 chambers when similar conditions are set and the same diet is fed to 5 pigs per chamber.

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee at the University of Illinois reviewed and approved the protocol for the experiment. Pigs used in the experiment were the offspring of

Line 359 boars and Camborough females (Pig Improvement Company, Hendersonville, TN).

Animals and Housing

A total of 30 pigs with an initial body weight (**BW**) of 38.3 ± 1.8 kg were randomly allotted to 6 calorimeter chambers with 5 pigs per chamber. Each air-tight chamber had a volume of 6.5 m^3 (1.3 m^3 per pig) and was equipped with a stainless steel wet-dry feeder with a capacity of 30 kg. An auxiliary drinker was also available in each chamber to ensure *ad libitum* access to water. The calorimetry chambers have ceiling and walls constructed from a wood-coated frame with sprayed-on plastic and galvanized steel modular slotted flooring with the animal contact surface made of a series of spaced triangular bars. The air supply duct and diffuser that provided the air exchange for the pigs are located in the chamber ceiling. Underneath each chamber, 4 stainless steel screens and 2 pans for total, but separate, collection of feces and urine, are installed. Room temperature and relative humidity in the chambers were kept at 23°C and 55% using a Parameter Generation Control unit. The air velocity was controlled by an air flow meter (AccuValve®) and maintained at 51 ± 3 m^3/h. Accuracy of chambers was tested using the propane burning method (Ismail, 2017).

Diets and Feeding

A common diet based on corn and soybean meal (**SBM**) was formulated (Table 4.1). All nutrients were included in concentrations that exceed requirements for 25- to 50-kg pigs (NRC, 2012; Table 4.2)**.** Feed was provided on an *ad- libitum* basis during 11 d followed by a 36-h fasting period. Water was available at all times.

Sample Collection

Pigs were housed in the chambers for 14 d and the amount of feed provided was recorded every time the feeder was refilled. The initial 5 d were the adaptation period to the chambers and

the experimental diet. At 0700 h on d 6, the gas analyzers started measuring gas concentrations and feces and urine were collected from d 6 to 11. On d 12, feed was removed from the feeders at 0700 h and a 36-h fasting period followed to determine fasting heat production (**FHP**). Feces, urine, and gas measurements from d 6 to 11 were considered samples from the collection period; whereas, urine and gases measured from d 13 at 0700 h to d 13 at 1900 h were considered samples from the fasting period. Following conclusion of the fasting period, feeders were refilled and in the morning of d 14, pigs were moved to growing-finishing facilities at the Swine Research Center.

Collected feces and 5% of the daily collected urine was stored at -20°C. To avoid N loss from the urine, 125 mL of 6*N* HCl were added to each urine pan every day. Chambers were opened every day to inspect feeders and animals, and to collect feces and urine. This period was not longer than 1 h, and the data from the gas analyzers obtained during this period and until the chamber reached a steady condition were disregarded in the calculation of heat production.

Chemical Analyses

The experimental diet was analyzed for crude protein (**CP**; method 990.03; AOAC Int., 2007) using a LECO FP628 Nitrogen/Protein apparatus (Leco Corporation, Saint Joseph, MI), and AA were analyzed on a Hitachi Amino Acid Analyzer, Model No. L8800 (Hitachi High Technologies America, Inc; Pleasanton, CA) using ninhydrin for postcolum derivatization and norleucine as the internal standard. Prior to analysis, samples were hydrolyzed with 6*N* HCl for 24 h at 110°C (method 982.30 E(a); AOAC Int., 2007). Methionine and Cys were determined as Met sulfone and Cysteic acid after cold performic acid oxidation overnight before hydrolysis (method 982.30 E(b); AOAC Int., 2007). Tryptophan was determined after NaOH hydrolysis for 22 h at 110°C (method 982.30 E(c); AOAC Int., 2007). Acid hydrolyzed ether extract (**AEE**)

was analyzed in the diet by acid hydrolysis using 3*N* HCl (Ankom HCl, Ankom Technology, Macedon, NY) followed by crude fat extraction using petroleum ether (AnkomXT15, Ankom Technology, Macedon, NY), and dry ash (method 942.05; AOAC Int., 2007). Total dietary fiber (**TDF**; method 991.43; AOAC Int., 2007) and dry matter (**DM**; method 930.15; AOAC Int., 2007) were also analyzed. Diet, feces, and urine samples were analyzed for gross energy (**GE**) using an isoperibol bomb calorimeter (Model 6400, Parr Instruments, Moline, IL). Benzoic acid was used as the standard for calibration. Urine was analyzed for N using the Kjeldahl method (method 984.13; AOAC Int., 2007) using a KjeltecTM 8400 apparatus (Foss, Eden Prairie, MN). Glucose, fructose, maltose, sucrose, stachyose, and raffinose were analyzed in the diet by extracting and quantifying the sugars using high-performance liquid chromatography with an autosampler (Alcott Chromatography Inc, Norcross, GA), a pump (Waters 510, Waters Corporation, Milford, MA), a column (Dionex Carbopac Pa1, Sunnyvale, CA), and a pulsed amperometric detector (Dionex) based on the procedure of Rocklin et al. (1998). Results were compared with known standards for glucose, sucrose, maltose, and fructose (Chem Service, West Chester, PA) and known standards for stachyose and raffinose (Sigma-Aldrich, St. Louis, MO) to determine concentrations of monosaccharides, disaccharides, and oligosaccharides.

Calculations and Statistical Analysis

Apparent total tract digestibility (**ATTD**) of gross energy (**GE**) was calculated following standard procedures (Adeola, 2001). Concentrations of DE and ME were calculated after determining energy excreted in feces and urine (Adeola, 2001). The concentration of O_2, CO_2, and CH_4 was averaged within days in the collection period. The average of O_2, CO_2, and CH_4 concentrations and urine N excretion were also calculated for the fasting period. Total heat production was calculated using the following equation (Brouwer, 1965):

$$THP = [(16.18 \times O_2 + 5.023 \times CO_2 - 2.17 \times CH_4 - 5.989 \times \text{urinary N})]$$

Urinary N was expressed in grams; whereas, gas concentrations were expressed in liters. Heat increment (**HI**) was calculated by difference between THP and FHP using the following equation (van Milgen and Noblet, 2003):

$$HI, kcal = THP - FHP$$

The HI, which is associated with the heat generated during digestion of the diet, can be expressed in kilocalories per kilogram of feed if the intake of feed is known. The concentration of NE was determined using the following equation (Li et al., 2018b):

$$NE, kcal/kg = ME - HI$$

To evaluate variation among chambers, a mean of the variables of the energy balance, ATTD of GE, DE, ME, HI, NE, and FHP was calculated and the standard deviation and coefficient of variation were also calculated. Data for consumption of O_2, production of CO_2, THP, NE, urinary N excretion, and respiration quotient (RQ) were also analyzed using the PROC MIXED of SAS. Homogeneity of the variance and normality were tested using the PROC UNIVARIATE statement of SAS (SAS Inst. Inc., Cary, NC). The model included the main effect of chamber with the experimental unit being the day. Therefore, there were 6 replicate days per chamber. An α level of 0.05 was used to assess significance among chambers.

RESULTS

The common diet used in this experiment was a corn-SBM based diet that is similar to diets often used in commercial farms for growing pigs. Values for CH_4 were negligible and were not taken into account for the calculation of THP or FHP. The mean initial body weight of pigs

was 38.30 kg with a variation below 5% (Table 4.3). The GE intake per day averaged 8,000 kcal with a coefficient of variation (**CV**) among chambers of 6.2%. Gross energy in feces and urine was 862.8 and 249.9 kcal/d, respectively; CV for GE in feces was 11.3%; whereas the CV for GE in urine was 23%. The ATTD of GE among chambers was 89.22% with a CV of 1.1%. Values for the concentration of DE and ME in the diet were 3,551 and 3,427 kcal/kg and the corresponding CV values were 1.1 and 2.0%, respectively. The THP of pigs was 3,596 kcal per day and the CV was 16.2%. When THP was expressed per kg of $BW^{0.60}$, the average value was 404.5 kcal per $kgBW^{0.60}$ with a CV of 17.4%. The FHP of pigs obtained in this experiment was 2,392 kcal per day, with a CV of 18.1%. When FHP was expressed per kg of $BW^{0.60}$, the average value was 269.4 kcal per $kgBW^{0.60}$ with a CV of 19.7%. Heat increment in pigs was 597.2 kcal per kilogram of feed intake and the CV calculated for HI was 12.3%. The NE in the diet used in this experiment was 2,829 kcal/kg with a CV of 4.2%. The amount of urinary N excreted by pigs in the fed state was 11.10 g per day and the CV was 7.0%. During the fasting period, pigs excreted 7.92 g of N per 12 h, and the CV was 7.8%. The RQ of pigs in the fed state was 0.99 with a CV of 2.8% whereas in fasting state was 0.72 with a CV of 3.8%.

Nitrogen excretion in urine was not different among the 6 calorimeter chambers during the experimental period (Table 4.4). The O_2 consumption, CO_2 production, and THP were greater ($P < 0.05$) for pigs located in chamber 4 compared with pigs located in the other 5 chambers. Pigs in chamber 5 also had greater ($P < 0.05$) values for O_2 consumption, CO_2 production, and THP than pigs in chambers 1, 2, 3, and 6. However, the values obtained in chambers 1, 2, 3, and 6 were not different. The value of RQ of pigs ranged from 0.957 to 1.032, the RQ of pigs in chamber 4 was greater ($P < 0.05$) than the RQ in chambers 1, 2, 5, and 6.

Likewise, pigs in chamber 3 had greater ($P < 0.05$) RQ than pigs in chambers 1 and 6, whereas pigs in chamber 6 had a lower ($P < 0.05$) RQ than pigs in chamber 2.

DISCUSSION

Calibration of the equipment and gas analyzers was performed prior to initiating the experiment. The equipment that controls temperature and humidity inside the chamber, and the positive pressure in the chambers, were monitored daily. Accuracy of the equipment was tested after the trial was completed with a validated test (propane test). Therefore, the differences observed in this research are likely a result of the specific behavior of the group of pigs in each chamber. All values in this experiment were obtained for groups of pigs, but are expressed as values per pig to facilitate the comparison with values in the literature. Results from the chemical analysis of the diet indicated that the diet was properly mixed and the objective of the experiment of using a diet that is similar to diets used in commercial farms was met.

The low CV observed for initial BW of pigs indicated homogeneity among chambers in terms of BW, which is important because of the impact of BW on THP of individually housed pigs and feed intake of group-housed pigs (Noblet et al., 1994b; Quiniou et al., 2000). Although the GE intake of pigs had a low CV (6.2%), the difference between the chamber with the greatest value (chamber 4) and the chamber with the lowest (chamber 6) GE intake was 1,377 kcal/day, which likely had an effect on other measurements. The CV for GE in feces and urine of pigs located in the 6 calorimeter chambers (11.3% and 23.0%, respectively) indicated that there is a greater variation in the GE that is excreted than in the GE ingested. This is likely because it is easier to accurately measure GE intake than GE excretion. Because the greatest and the lowest value for both GE in feces and urine was observed in pigs located in chamber 2 and chamber 3,

respectively, the differences in GE excretion by pigs may not be directly related to the GE consumed. However, regardless of the differences in GE ingested and the GE excreted by pigs, the values obtained for ATTD of GE and concentration of DE and ME had small differences among chambers as demonstrated by the CV of 1.1%, 1.1%, and 2.0%, respectively.

The average value for ATTD of GE obtained from the 6 chambers was greater than the one reported by Chastanet et al. (2007) for growing pigs fed a corn-SBM diet on *ad libitum* basis, but similar to the one reported by Liu et al. (2016) using a corn-SBM diet fed to growing pigs fed at 3, 4, and 5 % basis of their BW. Values for the concentration of DE and ME in the diet were close to those reported for a corn-SBM diet fed to 30-to 60-kg pigs (Dong et al., 2019).

The high CV obtained for both THP and FHP indicated that there were differences in these parameters among chambers. This is likely due to the fact that heat production is influenced by physical activity and feed intake (van Milgen et al., 1998; de Lange et al., 2006). The value for HI of pigs obtained in this experiment was lower than the value reported by Li et al. (2018b) using 45-kg pigs that were fed a corn-SBM diet. The observation that values for THP and FHP in pigs located in chamber 4 were the greatest among chambers may indicate that those pigs had a greater physical activity than pigs located in the other chambers. However, pigs in chamber 4 also had the greatest GE intake, which likely also contributed to the increased THP, FHP, and HI. This observation also indicates that the difference between data from this experiment and data from the literature may be a result of the group-housing of pigs and free access to feed.

Values for N excretion in urine obtained in this experiment are in agreement with values previously reported (Figueroa et al., 2000; Zhao et al., 2019) for pigs fed diets containing 18% CP. The differences in O_2 consumption, CO_2 production, and THP among chambers observed in

this study are likely a result of the differences in feed intake observed among chambers. However, the lowest values for O_2, CO_2, and THP are in agreement with data reported from previous research where a corn-SBM diet were used (Jaworski et al., 2016; Li et al., 2018b Li et al., 2018a), but average values were greater in this experiment compared with previous experiments. This is likely a result of the *ad- libitum* feeding used in this experiment, which results in greater feed intake than in previous experiments where restricted feeding was used.

Despite the high variation observed for THP, FHP, and HI values, the CV for the concentration of NE in the diet was low (4.2%). The NE of the diet used in this experiment was lower than values for 38-kg pigs reported by Lyu et al. (2019) using a corn-SBM diet with 17% CP. However, The NE of the diet used in the current experiment was greater that the values reported by Noblet et al. (1994b) for 45, 103 and 150-kg pigs. The greater NE in the current experiment may be a result of the group housing of pigs, which likely results in reduced loss of heat from the animals. However, the fact that there was 2% oil included in the diet used in this experiment likely also contributed to the greater NE compared with the value estimated by Noblet et al. (1994a).

Conclusion

Differences in THP, FHP, and HI of pigs were observed among chambers, which is likely due to differences in physical activity, due to the group-housed conditions. However, differences in feed intake as a result of pigs having free access to feed likely also contributed to the observed differences in THP, FHP, and HI. Nevertheless, the CV for NE among chambers was relatively low, which is the most important factor. However, future research will focus on identify sources of variability and determining how to reduce or eliminate these differences.

TABLES

Table 4.1. Composition (as-is basis) of the experimental diet.

Ingredient	%
Corn	67.45
Soybean meal, 48 % CP	28.00
Soybean oil	2.00
Limestone	0.87
Dicalcium phosphate	0.97
Sodium chloride	0.40
L-Lys-HCL	0.15
DL- Met	0.01
Vitamin-mineral premix[1]	0.15
Total	100.00

[1]The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kilogram of complete diet: vitamin A as retinyl acetate, 11,150 IU; vitamin D3 as cholecalciferol, 2,210 IU; vitamin E as selenium yeast, 66 IU; vitamin K as menadione nicotinamide bisulfate, 1.42 mg; thiamin as thiamine mononitrate, 1.10 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 1.00 mg; vitamin B12, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.6 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper chloride; Fe, 125 mg as iron sulfate; I, 1.26mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese hydroxychloride; Se, 0.30mg as sodium selenite and selenium yeast; and Zn, 125.1mg as zinc sulfate.

Table 4.2. Analyzed values for nutrients in the experimental diet (as-is basis).

Item	Common diet
Gross energy, kcal/kg	3,981
Crude protein, %	17.74
Dry matter, %	87.11
Starch, %	46.8
Glucose, %	0.28
Sucrose, %	2.88
Maltose, %	0.00
Fructose, %	0.20
Stachyose, %	1.50
Raffinose, %	0.59
Ash, %	4.37
Acid hydrolyzed ether extract %	5.35
Total dietary fiber %	12.6
Insoluble dietary fiber, %	11.4
Soluble dietary fiber, %	1.2
Total Indispensable AA, %	
Arg	1.14
His	0.47
Ile	0.79
Leu	1.51
Lys	1.09

Table 4.2. (Cont.)

Met	0.26
Phe	0.87
Thr	0.65
Trp	0.22
Val	0.87
Total Dispensable AA, %	
Ala	0.88
Asp	1.75
Cys	0.27
Glu	3.13
Gly	0.74
Pro	1.05
Ser	0.75
Tyr	0.61

Table 4.3. Apparent total tract digestibility (ATTD) of gross energy (GE) and concentration of digestible energy (DE), metabolizable energy (ME), and net energy (NE), total heat production (THP), fasting heat production (FHP), heat increment (HI), nitrogen excretion (N ex), and respiration quotient (RQ) in fed and fasting state in group hosed pigs.

	Chamber								
Item	1	2	3	4	5	6	Mean	SD	CV %
Initial BW, Kg	40.56	40.40	38.00	38.04	36.44	36.36	38.30	1.84	4.8
GE intake, kcal/d	8,384	8,020	7,897	8,723	7,631	7,346	8,000	499.01	6.2
GE in feces, kcal/d	861.9	997.4	753.3	960.1	784.2	819.9	862.8	97.55	11.3
GE in urine, kcal/d	218.5	350.6	180.8	253.9	264.1	231.4	249.9	57.38	23.0
ATTD of GE, %	89.72	87.56	90.46	88.99	89.72	88.84	89.22	1.00	1.1
DE, kcal/kg	3,571	3,485	3,601	3,542	3,571	3,536	3,551	39.90	1.1
ME, kcal/kg	3,468	3,311	3,510	3,427	3,434	3,411	3,427	67	2.0
THP, kcal/day	3,113	3,285	3,278	4,638	3,927	3,337	3,596	582	16.2
FHP, kcal/day	1,935	2,034	2,278	3,067	2,737	2,300	2,392	432	18.1
HI, kcal/kg	559.4	620.9	503.8	716.8	620.5	561.7	597.2	73.23	12.3
NE, kcal/kg	2,908	2,691	3,006	2,710	2,813	2,849	2,829	120	4.2
THP, kcal/kgBW$^{0.60}$	337.6	357.1	369.6	522.6	454.0	386.3	404.5	70.27	17.4
FHP, kcal/kgBW$^{0.60}$	209.8	221.1	256.9	345.6	316.5	266.3	269.4	53.06	19.7
N-ex fed state, g/d	10.96	12.01	10.14	11.98	11.13	10.39	11.10	0.78	7.0
N-ex fasting state, g/12h	7.29	8.36	7.09	8.68	8.06	8.01	7.92	0.61	7.8
RQ fed state	0.96	0.99	1.01	1.03	0.98	0.96	0.99	0.03	2.8
RQ fasting state	0.67	0.75	0.73	0.73	0.71	0.71	0.72	0.03	3.8

Table 4.4. Urinary nitrogen excretion (N-ex), oxygen (O_2) consumption, carbon dioxide (CO_2) production, total heat production (THP), respiration quotient (RQ) group-hosed pigs.

Item	Chamber							
	1	2	3	4	5	6	SEM	*P*-value
N-ex fed state, g/d	10.96	12.01	10.14	11.98	11.13	10.39	0.61	0.185
O_2, L/d	623.1^{c}	653.4^{c}	648.9^{c}	911.9^{a}	781.2^{b}	668.0^{c}	18.87	< 0.001
CO_2, L/d	600.3^{c}	647.0^{c}	653.3^{c}	941.6^{a}	768.6^{b}	640.7^{c}	22.49	< 0.001
THP, kcal/d	$3,113^{c}$	$3,285^{c}$	$3,278^{c}$	$4,638^{a}$	$3,927^{b}$	$3,337^{c}$	99.08	< 0.001
RQ fed state	0.964^{cd}	0.990^{bc}	1.005^{ab}	1.032^{a}	0.983^{bcd}	0.957^{d}	0.01	< 0.001

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CHAPTER 5: EFFECT OF DIETARY CRUDE PROTEIN CONCENTRATION ON HEAT PRODUCTION AND DIETARY CONCENTRATION OF NET ENERGY OF GROUP-HOUSED GROWING PIGS

ABSTRACT

A 26-d experiment was conducted to test the hypothesis that group-housed pigs fed a low CP diet will have reduced total heat production (**THP**) and increased net energy (**NE**) compared with pigs fed a high CP diet. Two experimental diets were formulated containing 21% or 14% CP. Thirty pigs (BW: 50.02 ± 6.4 kg) were randomly allotted to a 2-period switch back design with 2 diets and two 13-d periods with 5 pigs per chamber and 6 replicate chambers per diet. Diets were provided on an *ad- libitum* basis for 11 d per period, and the amount of feed provided was recorded every time the feeder was refilled. On d 12, feed was removed from the feeders and pigs had a 36-h fasting period to determine fasting heat production (**FHP**). The O_2 consumption and CO_2 and CH_4 production, as well as urine N, were measured during the fed and fasting period to calculate THP. Results indicated that there were no differences in GE intake, GE in feces, apparent total tract digestibility (**ATTD**) of GE, metabolizable energy (**ME**), THP, heat increment (**HI**), or NE between the 2 diets. Therefore, the hypothesis that diets with low CP provide more NE to pigs could not be confirmed.

Key words: group-housed pigs, heat production, net energy, protein concentration.

INTRODUCTION

Requirements for amino acids (**AA**) of monogastric animals may be partly met by supplying crystalline AA to the diets, which reduces urea synthesis and excretion (Canh et al., 1998). Growth performance of pigs is not affected by reducing dietary crude protein (CP) if all indispensable AA are supplied in the diet to meet requirements (Carpenter et al., 2004). In weanling pigs, reducing dietary CP will reduce the incidence of diarrhea (Le Bellego and Noblet, 2002), and a reduced CP in diets for pigs may result in reduced total heat production (**THP**) because of reduced energy needed for deamination of AA (Noblet et al., 2001). As a consequence, reduced CP inclusion in the diet may increase the concentration of net energy (**NE**) available for animal growth because of the energy saving caused by the reduced AA deamination (Zhao et al., 2019). However, under practical conditions it has been difficult to demonstrate that low CP diets that are fortified with crystalline AA result in greater NE than diets with greater concentrations of CP. Therefore, the objective of this research was to test the hypothesis that group-housed pigs fed a low CP diet will have reduced THP and increased NE compared with pigs fed a high CP diet.

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee at the University of Illinois reviewed and approved the protocol for the experiment. Pigs used in the experiment were the offspring of Line 359 boars and Camborough females (Pig Improvement Company, Hendersonville, TN).

Animals and Housing

A total of 30 pigs with an initial body weight of 50.02 ± 6.4 kg were allotted to a 2-period crossover design with 2 diets and two 13-d periods. The Experimental Animal Allotment

Program (Kim and Lindemann, 2007) was used for animal allotment. Pigs were housed in 6 calorimeter chambers. There were 3 replicate chambers per diet in each period and 5 pigs per chamber, for a total of 6 replicate chambers per treatment. The chamber was equipped with a stainless steel wet-dry feeder with a capacity of 30 kg. An auxiliary drinker was available in each chamber to ensure free access to water. Underneath the slatted floor, 4 stainless steel screens and 2 urine pans that allowed for total, but separate, collection of feces and urine were installed. The chamber temperature was kept at 23 °C and 55 % relative humidity, which were controlled by a temperature and humidity control unit (PGC, Parameter, Black Montain, NC). The air velocity was 68 ± 3 m^3/h, which was controlled using an air flow meter (AccuValve®, Accutrol Llc, Monroe, CT).

Diets and Feeding

Two experimental diets were formulated (Table 5.1) to contain different levels of CP. One diet was a normal protein diet with 21% CP and the other diet was a low protein diet with 14% CP. Vitamins and minerals were included in concentrations that exceed the requirements for 25 to 50 kg pigs (NRC, 2012; Table 5.2)**.** In the diet containing 14% CP, crystalline AA were included to meet requirements for indispensable AA. Feed was provided on an *ad- libitum* basis during the initial 11 d of the experiment, but, in the morning of d 12, feeders were emptied and pigs were fasted during the following 36 h. Water was available at all times throughout the experiment.

Sample Collection

Pigs were fed experimental diets for 11 d in each period, and the amount of feed provided was recorded every time the feeder was refilled. The initial 5 d were the adaptation period to the chambers and to experimental diets. At 0700 h on d 6, the gas analyzers started measuring gas

concentrations and feces and urine were collected from d 6 to 11. Urine was also collected during the 36-h fasting period when fasting heat production (**FHP**) was determined. The O_2 consumption and CO_2 and CH_4 production, as well as urine N, were measured during the fed and fasting periods. Collected feces and 5% of the daily urine collection were immediately stored at -20°C. To avoid N loss in the urine, 125 mL of 6*N* HCl were added to each urine pan every day, for a total of 250 mL per chamber. Chambers were opened every day to inspect feeders, and feces and urine were collected when chambers were open. This open period was no longer than 1 h per day, and the data from the gas analyzers obtained during this period and until the chamber reached the condition, set by the PGC unit, were disregarded in the calculation of heat production. At the end of the experiment, pigs were returned to the growing-finishing facilities at the Swine Research Center at University of Illinois.

Chemical Analyses

Experimental diets were analyzed for CP (method 990.03; AOAC Int., 2007) using a Leco FP628 Nitrogen/Protein apparatus (Leco Corporation, Saint Joseph, MI), and AA were analyzed on a Hitachi Amino Acid Analyzer (Model No. L8800; Hitachi High Technologies America, Inc; Pleasanton, CA) using ninhydrin for postcolum derivatization and norleucine as the internal standard. Prior to analysis, samples were hydrolyzed with 6*N* HCl for 24 h at 110°C (method 982.30 E (a); AOAC Int., 2007). Methionine and Cys were determined as Met sulfone and cysteic acid after cold performic acid oxidation overnight before hydrolysis (method 982.30 E (b); AOAC Int., 2007). Tryptophan was determined after NaOH hydrolysis for 22 h at 110°C (method 982.30 E(c); AOAC Int., 2007). Acid hydrolyzed ether extract (**AEE**) was analyzed in the diets by acid hydrolysis using 3*N* HCl (AnkomHCl, Ankom Technology, Macedon, NY) followed by crude fat extraction using petroleum ether (AnkomXT15, Ankom Technology,

Macedon, NY). Diets were also analyzed for ash (Method 942.05; AOAC Int., 2007). Total dietary fiber (**TDF**; method 991.43; AOAC Int., 2007), and dry matter (**DM**; method 930.15; AOAC Int., 2007) were also analyzed, and diets were analyzed for phytase activity (Phytex Method, Version 1; Eurofins, Des Moines, IA). Diets, feces, and urine samples were analyzed for gross energy (**GE**) using an isoperibol bomb calorimeter (Model 6400, Parr Instruments, Moline, IL). Benzoic acid was used as the standard for calibration. Urine was analyzed for N using the Kjeldahl method (Method 984.13; AOAC Int., 2007) on a KjeltecTM 8400 apparatus (Foss, Eden Prairie, MN). Diets were also analyzed for sugars and oligosaccharides. Glucose, fructose, maltose, sucrose, stachyose, and raffinose were analyzed in diets by extracting and quantifying the sugars using high-performance liquid chromatography with an autosampler (Alcott Chromatography Inc, Norcross, GA), a pump (Waters 510, Waters Corporation, Milford, MA), a column (Dionex Carbopac Pa1, Sunnyvale, CA), and a pulsed amperometric detector (Dionex) based on the procedure of Rocklin et al. (1998). Results were compared with known standards for glucose, sucrose, maltose, and fructose (Chem Service, West Chester, PA) and known standards for stachyose and raffinose (Sigma-Aldrich, St. Louis, MO) to determine concentrations of monosaccharides, disaccharides, and oligosaccharides in the diets.

Calculations and Statistical Analysis

Apparent total tract digestibility (**ATTD**) of GE was calculated (Adeola, 2001). Concentrations of digestible energy (**DE**) and metabolizable energy (**ME**) were calculated after determining energy excreted in feces and urine (Adeola, 2001). The concentration of O_2, CO_2, and CH_4 was averaged within the collection period. The average of O_2, CO_2, and CH_4 concentrations and urine N excretion were also calculated for each fasting period. Total heat

production during the collection period was calculated using the following equation (Brouwer, 1965):

$$\text{THP, kcal} = [(3.866 \times O_2 + 1.200 \times CO_2 - 0.518 \times CH_4 - 1.431 \times \text{Urine N})]$$

Urine N was expressed in grams; whereas, gas concentrations were expressed in liters. The FHP in the fasting period was calculated using the same equation. Heat increment (**HI**) was calculated by difference between THP and FHP, using the following equation (van Milgen and Noblet, 2003):

$$\text{HI, kcal} = \text{THP} - \text{FHP}$$

Although THP and FHP were calculated in kilocalories, HI is associated with the thermic increment that is generated by the diet. Therefore, HI can also be expressed in kilocalories per kilogram of feed if the intake of feed is known. The concentration of NE of the diets was calculated using the following equation (Li et al., 2018):

$$\text{NE, kcal/kg} = \text{ME} - \text{HI}$$

Data were analyzed using the PROC MIXED procedure (SAS Inst. Inc., Cary, NC). Normality of data was verified using the UNIVARIATE procedure. The model included diet as fixed effect and period as random effect with the experimental unit being the chamber. Mean values were calculated using the LSMeans statement and an α-value of 0.05 was used to assess significance among means.

RESULTS

All pigs consumed their corresponding diets without apparent problems and remained in the chambers until the end of the trial with no evident health issues. The initial body weight was not different between pigs fed high CP diet and low CP diet (Table 5.3). There was no difference

in the GE intake or the GE fecal output of pigs between the 2 dietary treatments. However, the GE output in urine was greater ($P < 0.05$) from pigs fed the high CP diet than from pigs fed the low CP diet. No differences in the ATTD of GE, the concentration of ME, or NE between dietary treatments were observed, but values for the concentration of DE in high CP diets were greater ($P < 0.05$) than values obtained in low CP diets.

There were no differences in values for THP, FHP, or HI, between pigs fed high CP diet or low CP diet (Table 5.4). However, when FHP was expressed in kcal/kgBW$^{0.60}$ pigs fed high CP diet had lower ($P < 0.05$) FHP than pigs fed low CP diet. Urinary N excretion in both the fed and the fasting state was greater ($P < 0.05$) from pigs fed the high CP diet compared with pigs fed the low CP diet. The respiration quotient (**RQ**) in the fed state was greater ($P < 0.05$) for pigs fed low CP diets than pigs fed high CP diets, but no difference in RQ of pigs between the 2 dietary treatments in the fasting state was observed.

DISCUSSION

The 36-h fasting period length was established in this experiment because it has been the most commonly used in experiments to determine FHP (Holmes and Breirem, 1974; Tess et al., 1984; van Milgen et al., 1998; Liu et al., 2014). Feces, urine, and gas measures from d 6 to 11 were considered samples from the collection period; whereas, urine and gas measured from d 13 at 0700 h to d 13 at 1900 h were considered gas from the fasting period. The concentration of GE and CP in the 2 experimental diets were in agreement with the objective of the experiment of comparing 2 isocaloric diets with a difference in CP concentration. Values for the concentration of DE in diets were similar to values reported in experiments using corn-SBM diets (Rundle, 2018). The observation that there was a difference in the concentration of DE between dietary

treatments was not expected because there were no differences in GE intake, GE in feces, or in the ATTD of GE between diets. Values obtained for the ATTD of GE in both diets were similar to those reported in other studies where corn-SBM diets were fed to growing pigs (Son et al., 2012; Liu et al., 2016), indicating that the ATTD of GE is not affected by the level of dietary protein. The lack of differences in the concentration of ME between dietary treatments was not expected due to the reduced concentration of urine energy (**UE**) observed in pigs fed the low CP diet compared with pigs fed the high CP diet. However, the UE in the urine for pigs fed the low CP diet was offset by the greater DE in the high CP diet, thus, resulting in ME values that were not different. Values for UE obtained in this experiment are close to values reported in previous studies (Kerr and Easter, 1995; Le Bellego et al., 2001; Noblet et al., 2001) that also showed a greater amount of GE in urine from pigs fed high CP diets compared with pigs fed low CP diets. This observation indicates that a high concentration of CP in the diet results in a high excretion of UE, which is likely due to the increased deamination of AA, and increased urea production.

Values obtained for THP in this experiment are greater than those reported by Holmes and Breirem (1974) but in agreement with those reported by others (Noblet et al., 1994; 2001; Liu et al., 2014). In these experiments, THP was expressed relative to the body weight of pigs because of the influence of body weight on THP (Noblet et al., 1994). However, differences in THP among experiments may be due to changes in the genetics of the pigs and improvements in nutrition that make animals grow faster. As a result, the metabolic rate and the heat produced by the animal increases (Brown-Brandl et al., 2004).

Values obtained for FHP in this experiment are in agreement with previous values (Tess et al., 1984; van Milgen et al., 1998) for 50-kg pigs, but greater than values reported for smaller pigs (Liu et al., 2014). Fasting heat production is believed to be an accurate approximation to the

animal requirement in terms of energy for maintenance. However, FHP may be influenced by the sanitary status of the facilities (Meer et al., 2019), the methodology used to determine FHP (Liu et al., 2014), the diet that was fed prior to the fasting period, and the animal composition and body weight (van Milgen et al., 1998). In the current study, a lower CP in the diet increased FHP of pigs. Previous data indicate that if animals are restricted in feed intake and individually housed, there is not impact of diet CP level on FHP (Noblet et al., 2001). Therefore, results in the current experiment may indicate that group-housed condition and the *ad- libitum* access to feed influence FHP in pigs.

An increase in urine excretion of N in pigs fed a high CP diet compared with pigs fed a low CP diet has been previously reported (Canh et al., 1998; Noblet et al., 2001). This confirms that because pigs do not store protein in the body and excess AA that are not used for synthesis of protein or other N containing compounds are deaminated and N is excreted in the urine in form of urea. The differences between the 2 dietary treatments in N excretion during the fasting state indicates that N excretion during the fasting period is affected by the diet that was consumed by the pigs during the fed state.

The RQ is the ratio of CO_2 produced to O_2 consumed by animals while they perform an activity. Values for RQ of pigs both during the fed state and the fasting state were in agreement with values reported for 50-kg pigs fed a corn-SBM diet (Jaworski et al., 2016). Respiration quotient values of 1 indicate that glucose was the preferred source of energy; whereas, values close to 0.7 indicate that lipids or protein were likely the subtrate of energy for the body (Noblet et al., 1993). The fact that pigs fed low CP diet had greater RQ than pigs fed high CP diet was not expected because it is believed that excess of nutrients will be used for lipogenesis in the body (Jakobsen and Thorbek, 1993), and increased protein in the diet will result in a greater

lipogenic rate in the body. However, the observation that the RQ of pigs during the fasting period was not different between the 2 dietary treatments indicates that this process may not be influenced by the dietary treatment offered during the fed period.

Individually housed pigs fed a high (18.9%) CP diet had a greater HI than pigs fed a low (12.3%) CP diet, which resulted in a reduced concentration of NE in the high CP diet compared with the low CP diet (Noblet et al., 2001). Based on these data and the fact that HI of pigs is increased as a consequence of the extra protein metabolism caused by the excess CP in the diet (Noblet, 1996), for the present study, the concentration of NE in the 2 experimental diets were expected to be different.

Results obtained for THP, FHP, and HI in pigs did not confirm the hypothesis for this experiment. Pigs fed diets that contain AA in quantities close to the requirement will not spend energy on metabolizing AA to carbon skeletons and amino groups (Brown-Brandl et al., 2004), and it was, therefore, believed that the NE of the low CP diet was greater than the high CP diet, but that was not the case. This observation contradicts values indicating that NE is greater in low CP diets than in diets with greater CP (Le Bellego et al., 2001). Values from this experiment also contradict the notion that the NE of corn is greater than the NE of SBM because corn was increased and SBM was reduced, in the low CP diet. However, it has been reported that ME of SBM is greater than published values, and sometimes also greater than ME values in corn (Sotak-Peper et al., 2015). The current results indicate that the NE of SBM may also be close to that in corn.

Conclusion

Values obtained for the concentration of DE, ME, and NE in the diet, as well as for THP and FHP of pigs housed in groups are in agreement with values reported for individually housed

pigs. However, the hypothesis that diets with reduced CP contain more NE than diets with greater CP could not be confirmed. It is, therefore, possible that modern commercial pigs utilize CP with a greater efficiency than older genotypes of pigs. However, additional research to verify that hypothesis is needed.

TABLES

Table 5.1. Ingredient composition of experimental diets, as-fed basis.

Ingredient, %	High CP (21%)	Low CP (14%)
Corn	62.60	80.99
Soybean Meal 48%	33.00	13.00
Calcium phosphate	0.97	1.37
Soybean oil	2.00	2.00
Limestone	0.87	0.75
Sodium chloride	0.40	0.40
Vitamin and mineral premix[1]	0.15	0.15
Phytase premix[2]	0.01	0.01
Phe	-	0.05
His	-	0.03
L-Lys-HCL	-	0.62
DL-Met	-	0.15
L-Thr	-	0.21
L-Trp	-	0.05
L-Val	-	0.14
L-Leu	-	0.08
Total	100.00	100.00

Table 5.1. (Cont.)

[1]The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kg of complete diet: vitamin A as retinyl acetate, 11,150 IU; vitamin D3 as cholecalciferol, 2,210 IU; vitamin E as selenium yeast, 66 IU; vitamin K as menadione nicotinamide bisulfate, 1.42 mg; thiamin as thiamine mononitrate, 1.10 mg; riboflavin,6.59 mg; pyridoxine as pyridoxine hydrochloride, 1.00 mg; vitamin B12, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.6 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper chloride; Fe, 125 mg as iron sulfate; I, 1.26mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese hydroxychloride; Se, 0.30 mg as sodium selenite and selenium yeast; and Zn, 125.1mg as zinc sulfate.

[2] Each diet contained 500 units of microbial phytase/kg included in a premix (Quantum Blue 5 G; AB Vista Feed Ingredients, Marlborough, UK).

Table 5.2. Analyzed composition, and calculated NE of experimental diets, as-fed basis.

	High CP	Low CP
GE, Kcal/kg	3,940	3,868
NE, Kcal/kg	2,512	2,631
CP, %	19.96	13.32
DM, %	87.49	87.06
Ash, %	4.69	3.88
AEE[1], %	4.99	4.99
Total dietary fiber, %	13.8	11.8
Soluble dietary fiber, %	1.6	11.4
Insoluble dietary fiber, %	12.2	0.4
Starch, %	45.5	57.7
Glucose, %	0.21	0.32
Sucrose, %	2.98	2.14
Maltose, %	0.00	1.15
Fructose, %	0.13	0.21
Stachyose, %	1.90	0.75
Raffinose, %	0.70	0.36
Total indispensable AA, %		
Arg	1.29	0.70
His	0.51	0.33
Ile	0.90	0.57
Leu	1.65	1.06

Table 5.2. (Cont.)

Lys	1.11	1.07
Met	0.28	0.30
Phe	0.98	0.63
Thr	0.72	0.60
Trp	0.25	0.16
Val	0.97	0.69
Total dispensable AA, %		
Ala	0.94	0.63
Asp	1.99	1.06
Cys	0.30	0.20
Glu	3.45	2.00
Gly	0.81	0.49
Pro	1.14	0.77
Ser	0.81	0.48
Tyr	0.68	0.39
Phytase activity FTU[2]/kg	450	450

[1]AEE= Acid hydrolyzed ether extract.

[2]FTU= One FTU is defined as the phytase activity that releases 1µmol per minute of P from sodium phytate at pH 5.5 and 37°C (Jones et al., 2010).

Table 5.3. Apparent total tract digestibility (ATTD) of gross energy (GE) and concentration of digestible energy (DE), metabolizable energy (ME), and net energy (NE) of diets containing high (21%) or low (14%) crude protein and fed to pigs.

Item	High protein	Low protein	SEM	*P*-value
Initial BW, Kg	50.41	49.64	5.76	0.619
IE, kcal/day	7,661	7,972	944.6	0.364
FE, kcal/day	884.2	949.0	86.32	0.197
UE, kcal/day	199.1	137.4	28.06	0.026
ATTD of GE, %	88.39	88.07	0.42	0.425
DE, kcal/kg	3,483	3,407	16.28	0.001
ME, kcal/kg	3,380	3,341	16.12	0.121
NE, kcal/kg	2,832	2,780	65.45	0.736

Table 5.4. Total heat production (THP), fasting heat production (FHP), heat increment (HI), urinary N excretion (N-ex), and respiration quotient (RQ) in fed and fasting states in diet containing high (21%) or low (14%) protein fed to pigs.

Item	High protein	Low protein	SEM	*P*-value
THP, kcal/day	3,584	3,404	356.41	0.530
THP, kcal/kgBW$^{0.60}$	340.3	327.4	16.30	0.588
FHP, kcal/day	2,493	2,306	225.32	0.306
FHP, kcal/kgBW$^{0.60}$	206.6	259.5	9.75	0.003
HI, kcal/day	548.1	541.9	70.98	0.952
Fed state				
N-ex, g/day	12.10	3.90	1.55	< 0.001
RQ fed state	1.06	1.12	0.053	0.030
Fasting state				
N-ex, g/12 h	6.00	4.20	0.50	0.004
RQ	0.72	0.77	0.041	0.230

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CHAPTER 6: CONCLUSIONS

The results of this work indicate that the Swine Calorimeter Unit (**SCU**) effectively controls the environmental condition in the 6 chambers, and the unit contains all the equipment needed to monitor gas exchange and heat production in group housed pigs kept under commercial productions. As a result, the SCU makes it possible to determine net energy (**NE**) in diets and feed ingredients that are offered on an *ad-libitum* basis.

Values obtained for THP and NE among chambers were comparable to values previously reported, however, the differences observed among chambers results in addition work being needed to identify the source of variation, Thus, studies will be conducted with the aim of reducing variability among chambers.

Results also indicate that previously published data indicating a reduced heat production in pigs fed low protein diets may not be accurate in modern genotypes of pigs fed diets on *ad-libitum* basis. Additional research in this area, is also needed.