THE EFFECTS OF RED MEAT OR SUGAR SUPPLEMENTATION DURING GESTATION

AND LACTATION ON MATERNAL REPRODUCTION, MATERNAL AND OFFPSRING

BLOOD PARAMETERS, AND OFFSPRING BODY COMPOSITION

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ABSTRACT

Using swine as a model for humans, this study was conducted to examine whether maternal consumption of added protein (red meat) or free sugar (glucose) throughout gestation and lactation was associated with maternal reproduction, maternal and offspring blood parameters, and offspring mature body composition. Maternal breed of sows of at least one year of age at breeding were utilized. At D-40 of gestation, sows were randomly assigned to one of four dietary supplementation treatments 1) Control (**CON**): corn-soy gestation diet; 2) Beef (**GB**): 4 oz cooked beef patty; 3) Sugar (**SUG**): sugar on a caloric equivalent to the beef patty; 4) Half sugar and half beef (SB)**:** a supplementation of a beef and sugar mixture. Fewest piglets farrowed per litter was seen in the CON sows when compared to GB, SUG or SB treatments ($P<0.04$), with no difference in total number farrowed between GB, SUG, and SB sows ($P>0.6$). Although GB sows had piglets with a lower average birth weight than CON sows ($P=0.02$) and no difference in average piglet growth during lactation ($P=0.17$) or in weaning weight ($P=0.08$). There were no ($P>0.05$) differences in traits measured in the sow (subcutaneous backfat depth, body weight, and energy balance). No treatment effect was seen in low-density lipoprotein (**LDLch**) ($P=0.31$); however, serum LDLch continued to decrease for SUG sows from early to late gestation. There were differences for high-density-lipoproteins (**HDLch**) with GB sows having the greatest HDLch levels when compared to CON and SUG during mid gestation ($P=0.05$ and 0.004, respectively). There were treatment differences seen during mid- and late gestation with GB sows having higher total cholesterol (**TOTch**) than SUG and CON sows ($P<0.05$). During mid gestation, TOTch was higher for GB than SB ($P=0.006$), resulting in lower TOTch for SB during late gestation ($P=0.08$). It can be concluded that the supplement provided during gestation and lactation, had a minimal effect on overall maternal reproduction, maternal

and offspring blood parameters, and on mature body composition of offspring. Positive outcomes for offspring and mothers cannot be expected if the supplementation is not needed.

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LIST OF ABBREVIATIONS

AA ....amino acid

ACOG .... The American College of Obstetricians and Gynecologists

AnGAP ....anion gap

ANPC .... Animal Nutrition and Physiology Center

BF .... Back fat

BUN .... blood urea nitrogen

CDC .... Center for Disease Control and Prevention

Cl.. chloride

CON .... supplementation of corn-soy gestation feed

D- ....day

DM ....diabetes mellitus

DP ....dressing percentage (%)

EBW.. eviscerated body weight

GB ....supplementation of 4 oz cooked beef patty

GDM .... gestational diabetes mellitus

GLU .... glucose

Hb... hemoglobin

Hct... hematocrit

HCW ....hot carcass weight

HDL .... high density lipoprotein

HDLch..high density lipoprotein cholesterol

HFS ....high fat and sugar diet

CHAPTER 1. INTRODUCTION

The global epidemic of obesity has been causally linked to the population's changes in diet and lifestyle. Obesity and type II diabetes mellitus are related and are one of the more commonly diagnosed problems continuously increasing in the Unites States (CDC, 2011). Obesity has become so prevalent that it is now considered to be a worldwide epidemic and a public health concern. The major determinates driving obesity are complex but clearly involve interactions with our environment, particularly related with food supply, eating behaviors, and genetics as well as public policies (Dixon, 2010). To ultimately maintain a healthy lifestyle, understanding the importance of proper diet and nutrition is essential.

Diabetes mellitus, either type I or type II, is considered to be the most common pre-existing medical condition in pregnant women (Yogev and Visser, 2009) and, in the United States, roughly 3-15 % of women develop gestational diabetes mellitus (**GDM**) during their pregnancy. Gestational diabetes mellitus is a state of impaired glucose intolerance that develops during pregnancy and is usually diagnosed in the 24th to 28th week of pregnancy. Additionally, as the prevalence of obesity continues to increase, so does the number of women at reproductive age who are either overweight or obese. This ultimately results in women entering pregnancy at higher weights which can result in complication during pregnancy as well as health concerns for both the mother and offspring post-partum. Obesity, type II diabetes mellitus, and GDM in pregnant women are associated with increased risks such as pre-eclampsia and delivery of *large-for-gestational* age infants (Patel et al., 2015). Fetal complications include congenital abnormalities, macrosomia, shoulder dystocia, still birth, growth restriction, and hypoglycemia (Watkins et al., 2003). Lastly, there is also increasing evidence for a causal role in the

development of childhood obesity in offspring of mothers who experienced GDM and obesity during pregnancy (Kim et al., 2012).

The condition of human pregnancy induces a physiological, insulin-resistant state in the mother (Sonagra et al., 2014). The purpose of insulin resistance adapted by the mother is to deliver enough quantity of nutrients to the growing fetus. There is roughly an additional 40 to 50 % increase in insulin resistance during pregnancy from pre-gravid conditions (Mason et al., 1994; Yogev and Visser, 2013). It is reported that pregnant women require an additional energy intake of 300 kcal/day over routine energy intake (Catalano et al., 1998) while the average glucose utilized by a growing fetus during the 3rd trimester reaches approximately 33 µmol/kg/min (Sivan et al., 1999). Thus, the development of insulin resistance serves as a physiological adaptation of the mother to ensure adequate carbohydrate supply for the rapidly growing fetus (Sivan et al., 1999; Sonagra et al., 2014).

Previous observational data and animal studies have highlighted the associations between *in utero* environmental exposures and increased susceptibility to obesity and related metabolic disorders post-partum and later in life. Animal models have been used to study the epigenetic mechanisms for the developmental programming of obesity. Samuelsson et al. (2008) showed that diet-induced maternal obesity in mice was associated with obesity, diabetes, fatty liver, hypertension, and behavioral changes in the offspring. Similar results have been seen in human studies where development of GDM during pregnancy is associated with an increased risk of several pregnancy and perinatal complications and may have the long-term health effects on both the mother and the offspring (Fan et al., 2006; Gilmore et al., 2015). The studies presented in this dissertation show the importance of nutritional research during the stages of gestation, lactation, and the early developmental stages of life. Examining the effects of added protein in the form of

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red meat (ground beef) or added sugar in the diet and their influence on maternal health can provide researchers with critical information about biological pathways and provide strategies to address causal relationships between maternal nutrition and offspring development.

A Western dietary pattern is characterized by an increased consumption of highly processed foods containing excess levels of fat, sodium, fresh and/or processed red meats, and refined sugars (Cordain et al., 2005; Astrup et al., 2008; Carrera-Bastos et al., 2011; Hintze et al., 2012). Additionally, a total western diet is generally lower in essential vitamins, minerals, and fiber that aid in the maintenance of optimal health. The two components of a total western diet that are of greater concern to consumers and health professionals is the increased intake of sugar and red meat. In Western societies, individuals are consuming large amounts of refined sugars, with the average American consuming between 67 to 77 kg of refined sugar per year (USDA, 2003). Contrary to popular belief, Americans seem to be consuming moderate amounts of red meat. It has been estimated that the average consumption of beef in America is roughly 1.7 oz/d (Zanovec et al., 2010), which falls well within the healthy eating patterns recommended by the 2010 *Dietary Guidelines for Americans*. For these reasons, it is important to examine and compare how added red meat and sugars in the diet effect overall health of an individual.

Excess intake of refined sugar combined with chronic low intakes of macro- and micronutrients can play a critical role in the development of chronic diseases including hypertension, obesity and metabolic syndrome, diabetes, kidney disease, and cardiovascular disease (Johnson et al., 2007; Hintze et al., 2012). Over the last 50 years, these chronic diseases have steadily increased (Misra et al., 2010; Carrera-Bastos et al., 2011). With the influence of nutritional guidance provided to consumers, the many key nutrients and high quality protein content contributed by red meat may be overshadowed (McNeill et al., 2012). Despite the unique

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nutrient package that comes with beef, consumption of beef has continued to decline among Americans (Davis and Lin, 2005). Today the food we consume is frequently scrutinized by consumers focusing on factors such as product composition, clean labels, product '*naturalness*', and issues with environment as well as sustainability (Troy and Kerry, 2010). Like any other product, meat is suffering from an undesirable image due to its alleged high fat content and consumption being consequential linked with specific health issues relating to cancer, heart disease, and obesity (Demeyer et al., 2008).

Objectives

Research is ongoing into which aspects of a Western diet trigger the effects of molecular changes in the offspring and, according to the developmental origins of health and disease hypothesis, most conditions that occur in adulthood originate in fetal life (Heindel et al., 2016). Given the potential modulatory effect of nutritional factors on disease, examining aspects of the TWD during pregnancy and/or lactation can provide vital information on the development of obesity-related metabolic disorders for the offspring and mothers.

The objectives of this study were to utilize swine as a model for humans to examine the role of how the maternal consumption of added ground beef, sugar, or a combination of the two supplemented in the diet during gestation and lactation would affect sow reproductive performance, early offspring growth and mature body composition, and blood chemistry parameters of the mother and offspring.

Hypothesis

High food quality, together with adequate macro- and micronutrient intake in pregnancy, is crucial for the health status of the mother and child. The maternal diet must provide sufficient energy and nutrients to meet the mother's usual requirements, as well as the needs of the growing

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fetus, and enable the mother to lay down stores of nutrients required for fetal development and for lactation. Therefore, the hypothesis of this study was that the supplementation of added protein (red meat) in the form of ground beef during gestation and lactation would have a positive impact on glucose metabolism, decrease maternal and offspring adiposity, increase the overall quality of milk production, and decrease the risk factors for obesity-related metabolic disorders in the offspring when compared to added dietary sugar consumed as a part of a maternal diet.

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CHAPTER 2. LITERATURE REVIEW

Because the physiology, reproductive system, organ development, and disease progression of swine are very comparable to humans (Lunney, 2007), swine have been widely utilized in research pertaining to diabetes and its co-morbidities (Schook et al., 2005; Vodička, 2005; Ibrahim et al., 2006; Swindle and Smith, 2008). Swine have also been widely used in cholesterol research (citations).

During pregnancy, it is essential that the diet provides an appropriate amount of energy and nutrients for the mother and fetus. The mother's nutritional status during the preconception period, during pregnancy, and lactation can affect the perinatal phase of pregnancy outcome (Osrin et al., 2000; Keen et al., 2003) and may also be related to the development of cardiovascular disorders, hypertension, and noninsulin dependent diabetes mellitus in her adult child (Barker, 1992). Gestational diabetes mellitus (**GDM**) can have a negative impact on fetal development as well as trigger intrauterine programming of diseases like obesity or diabetes in the offspring's later life (Martins et al., 2015). Red meat contains high biological value protein and important micronutrients that are needed for good health throughout life (Williams, 2007). Consuming red meat during pregnancy may help ensure nutritional requirements are met by the mother and developing fetus. Therefore, the first literature review below will cover the interrelationship of GDM, red meat consumption, and pregnancy.

Cholesterol is a white, insoluble, waxy substance that is transported through the blood via lipoproteins known as low-density lipoproteins (**LDL**) and high-density lipoproteins (**HDL**) (Lusis and Palukanta, 2008). The HDL form of cholesterol carries and removes excess cholesterol from the cells for delivery to the liver for disposal, while LDL cholesterol carries cholesterol from the liver to the rest of the body. Diabetes tends to lower HDL cholesterol levels

while increasing LDL cholesterol and triglycerides, which increases the risk for heart disease and stroke (Mooradian, 2009). This condition is commonly known as diabetic dyslipidemia. Studies have shown a link between insulin resistance, which is a precursor to T2DM, and diabetic dyslipidemia, atherosclerosis, and blood vessel disease (American Heart Association, 2019). Currently, the common practice is to utilize fasting blood draws for a lipid panel; however, there is a movement in human medicine towards non-fasting blood draws (Mora et al., 2008; Nigam, 2011). Therefore, the second literature review below delves more into cholesterol and its role in monitoring human health.

Diabetes Mellitus, Red Meat, and Pregnancy

Introduction

Diabetes mellitus (**DM**) can be referred to as a collective group of glucose intolerance syndromes (Rennard and Van Obberghen, 2006). The World Health Organization defines DM as a chronic, metabolic disease characterized by elevated levels of blood glucose (WHO, 2018), which can lead to other serious health complication affecting the cardiovascular system, nervous system, kidneys, and eyes. There are two main types of DM: the body either does not produce insulin (type I; **T1DM**) or cannot properly utilize the insulin it produces (type II; **T2DM**) and ultimately leads to systemic insulin resistance (**IR**, ADA, 2015). Both types of DM ultimately result in dysfunctional glucose metabolism and other metabolic disorders associated with absent or nonfunctional insulin.

Gestational diabetes mellitus is defined as glucose intolerance expressed in variable severity with onset or first recognition during pregnancy (Baynest, 2015). Additionally, about half of women with a history of GDM go on to developing T2DM within five to ten years after delivery (Herath and Herath, 2017; Cho et al., 2017). In 2017, an estimated 204 million women

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worldwide ages 20-79 years old were living with some form of DM and 1 in 3 of those women

with DM were of reproductive age (International Diabetes Federation, 2019). The International

Diabetes Federation also stated that, of the 21.3 million live births that occurred in 2017, there

was some form of hyperglycemia present during pregnancy and, of those instances where

hyperglycemia occurred, 81.5 % were due to GDM (Cho et al., 2018). Given the tremendous

increase in the prevalence of adolescent obesity and T2DM in the United States, long-term

implications for developing countries are important. As developing areas strive to meet the

nutritional demands of women of reproductive age, there is the potential that adolescent T2DM

and obesity will continue to increase worldwide. This literature review will consist of two parts:

first, it will examine and discuss the pathology, diagnosis, and treatment of diabetes, specially

focusing on GDM and how it impacts overall health of the mother and their offspring; second, it

will examine the role of red meat in the diet in relation to pregnancy.

Pathophysiology

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia

resulting from defects in insulin secretion, insulin action, or both (ADA, 2014). Type I DM is a

disorder in which the immune system attacks the insulin-producing beta-cells of the pancreas and

is most often diagnosed in children and adolescents (Atkinson and Eisenbarth, 2001; Bluestone

et al., 2010). This autoimmune destruction of beta-cells alters insulin secretion, ultimately

leading to an insulin deficiency. Absolute insulin deficiency has many physiological

consequences, including the disruption of glucose uptake into muscle and adipose cells and the

absence of an inhibitory effect on hepatic glucose production, lipolysis, and ketogenesis, with

extreme insulin deficiency leading to elevated free fatty acid levels and diabetic ketoacidosis

(Delaney et al., 2000; Eldrisi et al., 2006). Lastly, muscle wasting observed in patients with

uncontrolled T1DM results from the failure to stimulate amino acid uptake and protein synthesis and inhibit protein degradation (Newsholme, 2001). In addition to the dysfunction of the pancreatic beta-cells, type I diabetics also have abnormal function of pancreatic alpha-cells, resulting in an excessive secretion of glucagon (Baynest, 2015). Ultimately, in T1DM, circulating insulin is very low or absent, plasma glucagon is elevated, and the pancreatic beta-cells fail to respond to all insulin-secretory stimuli (Van Belle, 2011).

Type II DM is a progressive disease associated with hyperglycemia (Stratton et al., 2000) that increases the risk for developing other health related concerns. The disease course of T2DM is multifactorial and includes both genetic and environmental elements that alter insulin sensitivity. These physiological alterations influence the function of the pancreatic beta-cells as well as muscle, liver, and adipose tissues (Scheen, 2003). Type II DM is associated with the condition of IR. This means that the pancreatic beta-cells are producing the hormone insulin; however, the target tissues do not recognize insulin. If insulin is not recognized by its target tissue, it cannot function as an endocrine hormone responsible for serum glucose clearance, glycogen synthesis (intracellular glucose storage), secondary messenger gene expression, and protein synthesis (Steppan et al., 2001; Melmed et al., 2015). Normally, beta-cells respond to IR by increasing their production of insulin to meet the requirements of tissues. Fonseca (2009) suggested that the development of T2DM was caused by the failure of the pancreatic beta-cell to effectively compensate for IR. Because T2DM can have a gradual onset, having a fasting plasma blood glucose concentration between 100 and 125 mg/dL is considered as having impaired insulin activity and the individual would be considered to have prediabetes (ADA, 2017). People with prediabetes have an increased risk of developing T2DM, heart disease, and stroke. Studies have shown that people with prediabetes who lose weight and increase their physical activity can

prevent or delay T2DM and, in some cases, return their blood glucose levels to normal (Sherwin et al., 2004; Khaodhiar et al., 2009; CDC, 2011; Wilding, 2014).

Insulin requirements are usually higher during pregnancy (Homko et al., 2001; Kaaja and Rönnemaa, 2008). This occurs because the normal hormone production and weight gain during pregnancy can increase IR. The pathophysiology of GDM involves abnormalities of insulin-sensitive tissue as well as beta-cell sensing of glucose. Women who develop GDM have been shown to have beta-cell dysfunction and IR prior to pregnancy (Kautzky-Willer et al., 1997; Wallace et al., 1997) and the condition becomes exacerbated due to the immune reaction in response to the developing fetus. While this is most commonly the cause of GDM, the American Diabetes Association defines GDM as "*any degree of glucose intolerance with onset or first recognition during pregnancy*" (ADA, 2017), which would include the recognition of T1DM or T2DM during pregnancy (Kaaja and Rönnemaa, 2008). Being diagnosed with GDM during pregnancy is a strong risk factor for an individual to develop DM later in life (Lauenborg et al., 2004). At risk women should receive an oral glucose tolerance test at least 6 weeks after parturition and be reclassified as having diabetes, normal glucose tolerance, impaired glucose tolerance, or impaired fasting glucose (Baynest, 2015).

Insulin resistance is a key component of T2DM and GDM and is defined as a state that requires more insulin to obtain the biological effects achieved by a lower amount of insulin in the normal state. Any defects in the insulin signaling cascade can cause IR (Kadowaki et al., 2006), including down regulation of receptors on the surface of target cells, making the cells less sensitive to insulin. The two most common conditions associated with insulin receptors being down-regulated are obesity and diabetes (Kahn and Flier, 2000; Steppan et al., 2001). Elevated levels of the hormone insulin in the blood trigger down regulation of the associated receptors.

When insulin binds to its receptors on the surface of a cell, the hormone receptor complex undergoes endocytosis, which provides a pathway for degradation of insulin as well as for regulation of the number of receptors on the cell surface (Carpentier, 1994).

Diagnosis and Treatment

The identification of DM or prediabetes by screening allows for earlier intervention, which can potentially reduce future health complication that co-exist with diabetes. Diabetes mellitus may be identified anywhere along the spectrum of clinical scenarios and detection in the early stages of the development of T2DM is critical for implementation of preventative measures. Three tests are used to screen for and diagnose DM. They are; A1C (glycated hemoglobin), fasting plasma glucose concentration, and oral glucose tolerance test. Normal, prediabetic, and diabetic values are given in Table 1.1 and a more detailed description of each test follows.

Table 1.1. Classification of diabetes using the different methods of diagnosis.[a]

Diagnosis	A1C (percent)	Fasting plasma glucose[b]	Oral-glucose tolerance test[bc]
Normal	Below 5.7	99 or below	139 or below
Prediabetes	5.7 to 6.4	100 to 125	140 to 199
Diabetes	6.5 or above	126 or above	200 or above

[a] Table adapted from information from ADA, 2016.
[b] Glucose values are in milligrams per deciliter
[c] At 2 h post-consumption of 75 g of glucose in a liquid solution; To diagnose gestational diabetes, health care professionals increase the glucose in the solution and use different classification values

The A1C test is the primary test used for diabetes management. It is a blood test that provides information about average levels of blood glucose for an individual over the past 3 months (Malkani and Mordes, 2011). This test is based on the attachment of glucose to hemoglobin, which means the hemoglobin has become glycosylated. When glucose concentrations are higher than the normal range, glucose attaches to proteins in the body,

specifically hemoglobin, which is part of the red blood cells. As a person's blood sugar becomes higher, more of the person's hemoglobin becomes glycosylated (Zhang et al., 2012). The glucose remains attached to the hemoglobin for the life of the red blood cell, or about 2 to 3 months. The A1C test result is reported as a percentage of glycated hemoglobin relative to non-glycated hemoglobin. A higher percentage is indicative of prolonged blood hyperglycemia (The National Institute of Diabetes and Digestive and Kidney Diseases, 2018). A normal A1C percentage for healthy humans is lower than 5.7 %. An A1C percentage between 5.7 and 6.4 % would be considered prediabetic, while any percentage over 6.5 % would result in a DM diagnosis (Mann et al., 2010; Nowicka et al., 2011).

The fasting plasma glucose blood test measures blood glucose concentration at a single point in time. The optimal time to perform this analysis is in the morning after fasting has occurred for at least 8 h with nothing to eat or drink except sips of water (Hu et al., 2010; Sacks, 2011). Normal fasting plasma glucose levels should read less than 100 mg/dL. While a diagnosis of prediabetes will range from 100 mg/dL to 125 mg/dL, a fasting plasma glucose concentration more than 126 mg/dL on two or more tests confirms a diabetes diagnosis (Nathan et al., 2009). Another form of plasma glucose testing is called a random or casual plasma glucose test. This is the simplest test and does not require fasting before taking the test.

The oral glucose tolerance test for diagnosis of T2DM is a two-hour test that monitors blood glucose levels before and 2 h after drinking a glucose-water mixture. An initial fasting blood sample is taken to determine the fasting blood glucose level. Then the patient would consume 237 mL of a glucose solution containing 75 g of glucose. At 2-h post-consumption, a blood sample will be taken again to determine blood glucose levels. A 2-h blood glucose concentration between 140 and 199 mg/dL may result in a prediabetes, or glucose intolerance

diagnosis while a concentration of 200 mg/dL or greater may result in a T2DM diagnosis, depending on other factors (Mayo Clinic, 2018).

For diagnosis of GDM, a Glucose Challenge Screening is performed between 26 and 28 weeks of gestation. For women at high risk of developing GDM, this test may be performed earlier. For the Glucose Challenge Screening, pregnant women are asked to drink a glucose drink (237 mL containing 75 g glucose) and blood is collected 1-h post-consumption. If the results of this test are positive or border-line, a 3-h Glucose Tolerance Test may be performed (American Pregnancy Association, 2018; Mayo Clinic, 2018). For the Glucose Tolerance Test, a fasting blood draw will be used to determine the baseline fasting blood glucose concentration. After the initial blood draw, the pregnant woman would drink a 237-mL glucose drink containing 100 g of glucose. Blood will be drawn and tested at 1, 2, and 3 h post-consumption. A diagnosis of GDM will occur if fasting blood glucose levels are greater than 95 mg/dL, one-hour levels are greater than 180 mg/dL, two hour levels are greater than 155 mg/dL, or three hour levels are 140 mg/dL or higher (American Pregnancy Association, 2018; Mayo Clinic, 2018a).

The metabolic conditions that lead to a diagnosis of DM are complex and can vary from one individual to another. For some, a blood glucose test may indicate a diagnosis of diabetes even though an A1C test does not; the reverse can also occur. People with contradicting test results may be in an early stage of the disease, where blood glucose levels have not risen to a point of accurate diagnosis on every test. Health professionals may find it prudent to run multiple tests to confirm a diabetes diagnosis, especially if other risk factors such as obesity are present (NIDDK, 2019).

Treatment for DM, regardless of the type, requires the individual to track blood sugar levels and combine proper nutrition, physical activity, and, in some cases, medication. Each

treatment plan is tailored to the individual and can be adjusted based on what is eaten, the amount of exercise, and for times of stress and illness (ADA, 2017).

Global Prevalence of Diabetes Mellitus

Prediabetes and Diabetes Mellitus

According to the Center for Disease Control and Prevention (**CDC**), an estimated 33.9 % of United States adults aged 18 years or older (84.1 million people) had prediabetes in 2015, based on their fasting glucose levels (CDC, 2017). Additionally, the prevalence of prediabetes is increasing worldwide, and experts have projected that more than 470 million people will have prediabetes by 2030 (Tabák et al., 2012). The World Health Orginization *Global Report on Diabetes 2017* revealed that the number of adults living with diabetes has almost quadrupled from 108 million in 1980 to 422 million adults with diabetes in 2014 (WHO, 2017). The global prevalence (age-standardized) of diabetes has nearly doubled since 1980, rising from 4.7 % to 8.5 % of the adult population (WHO, 2017). The prevalence of this disease has been dramatically increasing worldwide due to the increased diagnosis of T2DM (Menke et al., 2015). In the United States, T1DM, commonly known as juvenile-onset diabetes, represents approximately 10 % of all cases of DM while T2DM, commonly known as adult-onset diabetes, represents the remaining 90 % (Cefalu, 2006).

Some population groups are more genetically predisposed to develop diabetes during their lifetime. According to the *National Diabetes Statistics Report of 2017*, African American, Asian American, Hispanic, and Native American populations have a higher risk for developing T2DM when compared to non-Hispanic white Americans (CDC, 2017). According to the CDC (2017), there was an estimated 1.5 million new cases of diabetes (6.7 per 1,000 persons) diagnosed among adults in the United States aged 18 years or older in 2015. Of these cases, Non-

Hispanic blacks (9.0 per 1,000 persons) and people of Hispanic origin (8.4 per 1,000 persons) had a higher age-adjusted incidence compared to non-Hispanic whites (5.7 per 1,000 persons) during 2013-2015 (CDC, 2017).

This public health concern and the co-morbidities associated with it have been consistently increasing in diagnosis worldwide over the past 20 years. The increased diagnosis has occurred in all countries, populations, and across every economic status. It is well known that prediabetes and IR commonly coexist with obesity and it is estimated that 80 % of diabetics are overweight and 49 % are classified as obese (Nguyen et al., 2011). Lastly, DM was the seventh leading cause of death in the United States in 2015. This finding is based on 79,535 death certificates in which DM was listed as the underlying cause of death (crude rate, 24.7 per 100,000 persons) as well as being listed as any cause of death on 252,806 death certificates in 2015 (crude rate, 78.7 per 100,000 persons; Ogurtsova et al., 2017).

Gestational Diabetes

The global prevalence of GDM has increased by more than 30 % within the last two decades (Ferrara, 2007; Anna et al., 2008; Zhu and Zhang, 2016). According to the 2015 report from the International Diabetes Federation, there were about 20.9 million (16.2 %) live births affected by hyperglycemia in pregnancy and, of those cases, 85.1 % were due to gestational diabetes. It is important to note that approximately 87 % of hyperglycemic pregnancies were diagnosed in third world countries (Mahtab and Bhowmik, 2016). The global prevalence of GDM was recently estimated in a study of 36 countries (Melchior, 2017). The Middle East and North Africa had the highest prevalence of GDM with a median estimate of 12.9 % (range: 8.4-24.5 %), whereas Europe had the lowest prevalence (median: 5.8 %; range: 1.8-22.3 %).

The prevalence of GDM has increased steadily in the United States (Albrecht et al., 2010). Past studies have provided population-based prevalence estimates of GDM using both birth certificates and the Pregnancy Risk Assessment Monitoring System; a questionnaire completed by the mothers (DeSisto et al., 2014). Correa et al. (2015) conducted a study using the Nationwide Inpatient Sample to describe the trends in GDM prevalence among delivery hospitalization in the United States from 1993 to 2009. This study showed the prevalence of GDM increased from 3.09 to 5.57 per 100 deliveries over this period of time.

The American Diabetes Association (ADA, 2016) categorizes GDM rates by race/ethnic background as follows: non-Hispanic Whites (7.4 %), Asian Americans (8.0 %), Hispanics (12.1 %), non-Hispanic Blacks (12.7 %), and American Indian/Alaskan Natives (15.1 %). A study from Thorpe et al. (2005) reported trends specific to GDM using birth certificates from 1990 to 2001 and saw a significant increase in GDM across all major racial/ethnic groups except non-Hispanic White women. Two studies from the Kaiser Permanente health systems in the United States (Ferrara et al., 2004; Dabelea et al., 2005) examined incidence rates in pregnant women of multiple ethnicities for GDM between 1991 and 2002. Both studies showed that incidence rates of GDM in the United States rose over time, from roughly 4 % to more than 6 %. When examing ethincity, GDM was most common among people of Asian ancestry or Hispanic ethnicity and least common in people of European ancestry (Ferrara et al., 2004; Dabelea et al., 2005). Additionally, a report from Kaiser Permanente Southern California health system which spanned from 1995-2009 indicated that the overall incidence rate of GDM was 10 %, with higher rates in Asian (17 %) and Hispanic (11 %) women and lower rates in non-Hispanic white (7 %) and black (7 %) women (Xiang et al., 2011).

Along with race, age plays a factor in development of GDM and complications for the infant. Previous studies have shown strong associations between increased age and the development of GDM (Makgoba et al., 2011; Kampmann et al., 2015; Schoenaker and Mishra, 2017). Another complication with age and GDM is that women who are advancing in age can be at risk for increased infant mortality and stillbirth (Khalil et al., 2013). Rosenstein et al. (2012) examined the optimal time for delivery to improve perinatal outcomes and to understand the risks of stillbirth and neonatal/infant morbidity and mortality in relation to GDM. The risk of stillbirth increased continuously with gestational age in women with and without GDM rising to its highest level at 42 wk of gestation (Rosenstein et al., 2012). The overall risk of stillbirth at 36-42 wk of gestation was higher in women with GDM when compared with women without GDM (17.1 vs. 12.7 per 10,000 deliveries; Rosenstein et al., 2012).

Gestational Diabetes and Fetal Development

The identification and occurrence of fetal programming has led to the recent theory of fetal origins of adult disease. The "Barker hypothesis" (Hales and Barker, 1992; Hales and Barker, 2001) has suggested that embryonic and fetal life developmental changes in the structure of a number of organs has a critical impact on these organs' associated functions. These alterations in organ development (and subsequent function) determine the set point of physiological, and metabolic responses that carry into adulthood. Therefore, any stimulus during critical periods of fetal development may result in adaptations that produce permanent structural, physiological and metabolic changes that predispose an individual to cardiovascular, metabolic and endocrine disease in adult life (Hales and Barker, 1992; Hales and Barker, 2001). Lucas (1991) and Kwon and Kim (2017) suggested that fetal programming can affect diseases into adulthood; that is, the body's "memory" of nutrition during the early stages of development

translates into a pathology that determines susceptibility to future disease. Therefore, it is extremely important to understand the impact that GDM has on fetal development as it impacts the offspring for life.

Throughout pregnancy, the maintenance of optimal blood glucose concentration is very important for mother and infant health. The placenta provides oxygen and nutrients to the fetus and also aids in the elimination of wastes; therefore, the maternal glucose can cross the placenta to the fetus (Vambergue, 2011). Around 13 weeks of gestation, the fetus begins to make its own insulin and, if there is constant exposure to high levels of glucose, it is as if overeating were occurring, where the baby will produce more insulin in response to the excess glucose, resulting in weight gain and subsequent increase in size (Reece et al., 2009). Maternal nutrition plays a critical role in fetal growth and development because under- and over-nutrition during gestation can alter expression of fetal genomes resulting in lifelong consequences. The fetus of pregnant women who have DM or GDM are exposed to excessive amounts of glucose and amino acids (**AA**), which stimulate growth through the overproduction of insulin by the fetal pancreas (Patel et al., 2015).

There is growing evidence that GDM increases the risks of both short- and long-term health consequences for the fetus and mother. The most significant is a predisposition to the development of metabolic syndrome and T2DM (Reece, 2010). Epidemiological data has also suggested that women with GDM can deliver children with increased adiposity, insulin resistance, possess an increased risk of type 2 diabetes, and susceptibility to cardiovascular metabolic disorders; which, in turn, leads to transgenerational transmission of diseases (Jones, 2001; Reece, 2010; Murthi et al., 2019). In pregnancies complicated by GDM, increased placental production of pro-inflammatory cytokines interleukin-1 beta and tumor necrosis factor-

alpha have been observed (Marseille et al., 2008). Coughlan et al. (2001) found that mothers with GDM release greater amounts of tumor necrosis factor-alpha when exposed to high glucose concentrations. Therefore, it is suggested that the increased glucose levels can cause overexpression of pro-inflammatory cytokines, which may have negative impacts on placental development in GDM pregnancies.

A fetus with a birthweight exceeding 4000-4500 g is referred to as macrosomic (Reece et al., 2009). Neonatal birth weights have been strongly associated with glucose concentration in women with GDM (Langer et al., 1989). Increased insulin concentration in both fetal blood and amniotic fluid correlates with an increased rate of occurrence of fetal macrosomia (Reece et al., 2009). Law et al. (2019) examined the roles of glucose on the development of large for gestational age (LGA) infants in women who possessed GDM. This study found that maternal glucose levels were higher in women who delivered LGA infants (111.6 mg/dL vs. 104.4 mg/dL). Additionally, Law et al. (2019) found that mothers of LGA infants possess higher glucose concentrations overnight compared with mothers without LGA, suggesting that nocturnal glucose control may help reduce rates of LGA in women with GDM. Furthermore, LGA infants have an increased risk of injury, such as shoulder dystocia and newborn asphyxia, during vaginal birth delivery (Gottlieb and Galan, 2007; Henriksen, 2008; Reece et al., 2009).Lastly, it has also been shown that the offspring of mothers with GDM had a significantly higher birthweight on average than their counterparts whose mothers did not have GDM (Baptiste-Roberts et al., 2012).

Macaulay et al. (2018) assessed longitudinal fetal growth and neonatal birth measures among Black African babies exposed to GDM. Repeated ultrasound measures showed that the exposure to GDM was associate with increased fetal growth as early as 16 to 18 weeks of

gestation. Additionally, male fetuses exposed to GDM had larger abdominal circumferences when compared to females. The Pima Indians, a group of Native Americans in the western part of the USA and northwest Mexico, have one of the highest rates of GDM in the world (Dabelea et al., 2000). Research on this population has shown that diabetes during pregnancy is a major risk factor for T2DM and hyperglycemia in the offspring. Pima children exposed to GDM during pregnancy were more obese and had a higher prevalence of T2DM at 25-34 years of age when compared to children born to Pima women without diabetes (Dabelea et al., 2000). Additionally, exposure to hyperglycemia in utero has caused 40 % of Pima women children (5-19 yr) to develop T2DM (Dabelea et al., 2000; Reece et al., 2009).

Gestational Diabetes and Offspring Growth

The prevalence of childhood obesity has increased to over 40 % these past 20 years – with affiliated increases in childhood T2DM. It has also been shown that in regard to siblings, offspring born after the mother developed GDM orT2DM had a higher BMI and a higher risk of developing DM than offspring born before their mother developed DM (Dabelea et al., 2000). In a study by Wang et al. (2018) maternal abnormal glucose was associated with a higher risk of childhood overweight at 1-6 yr of age when compared to those born to mothers who had normal glucose levels during pregnancy. In addition, Baptiste-Roberts et al. (2012) showed that offspring of mothers with GDM were heavier in terms of weight and z-score of BMI at ages 4 and 7 compared to offspring of mothers without GDM. Lastly, compared to offspring of mothers without GDM, there was a higher proportion of overweight at ages 3, 4 and 7 among offspring of mothers with GDM; with GDM exposed children having 61 % higher odds of being overweight by the age of 7 (Baptiste-Roberts et al., 2012).

When examining the relationship between GDM and overweight/obesity in adolescent offspring, ages 9–11 yr, it was found that there was an increased risk with those individuals born of mothers who had maternal GDM; but not with pre-existing T2DM (Lawloe et al., 2010). Another study showed that during adolescence and by the by the age of 17 years, the progeny of women with GDM had higher BMI scores (Tsadok et al., 2011). Additionally, it has been shown that the offspring of women with GDM had an 8-fold increased risk of diabetes or prediabetes due to maternal diagnosis of GDM or a pre-existing T1DM and T2DM (Clausen et al., 2008). Data from this study also indicated that intrauterine hyperglycemia was associated with T2DM and/or pre-diabetes in adult offspring, with the prevalence of T2DM and pre-diabetes being 21 % in the offspring of mothers with GDM, 11 % in those whose mothers had pre-gestational T1DM, and 4 % in the offspring of mothers with no diabetes at 22 yr of age (Clausen et al., 2008).

Red Meat, Nutrition, and Pregnancy

Pregnancy is a period of intense fetal growth and development as well as a time of maternal physiological change. It is important to ensure proper macro- and micronutrients are being consumed during pregnancy for the overall health of the mother as well as the offspring. Ultimately, under- or over-nutrition can be associated with many adverse pregnancy outcomes to both the mother and offspring. It is well known that maternal nutrition may influence the outcomes of the pregnancy itself and future childhood development. The first trimester is the critical period for development and differentiation of various organs, whereas the diet consumed later in pregnancy may be more important for overall fetal growth and brain development (IOM, 1990; Piirainen et al., 2006). Fetal under- and over-nutrition can lead to permanent changes of fetal metabolic pathways and, thereby, increase the risk of childhood and adolescent diseases. The developmental model for the origins of disease hypothesized by Barker and Thornburg

(2013a) indicates that fetal environment triggers epigenetic modifications that impact gene expression in an effort to develop offspring that are adapted to that specific environmental circumstance. In terms of evolution, this can be a beneficial adaptation; however, activation or inactivation of developmental genes can also influence development of disease later in the lives of children and as adults.

Women who routinely eat three meals a day that include a variety of foods representing all food groups are likely to meet the United States Department of Agriculture (USDA) recommended daily allowance for most nutrients (Yunsheng et al., 2003). Since the number of additional calories required for a typical pregnancy can vary, women should focus on increasing intake of high-quality, nutrient-dense foods and attempt to limit intake of empty-calorie foods and beverages (IOM, 2011). With that said, even consumption of nutrient-dense foods recommended by the United States Department of Agriculture's *Food Patterns* may not meet nutrient goals for iron, vitamin D, and choline during pregnancy (Park and Eicher-Miller, 2014).

Beef is a nutrient dense food that contributes many important nutrients such as high-quality protein, conjugated linoleic acid, B-vitamins, choline, zinc, and iron to the diet. Several studies (Biesalski, 2005; Zanovec et al., 2010; O'Neil et al., 2011) have concluded that beef can provide multiple nutritional benefits during pregnancy. Consuming red meat during pregnancy can help ensure nutritional requirements are met by the mother and developing fetus. Understanding how to properly incorporate meats and other foods of animal origin during gestation may be the key to fetal development that reduces the chances of obesity and obesity-related metabolic disorders developing later in life.

Protein and Amino Acids

A developing fetus needs an adequate amount of protein, especially during the second and third trimesters. In order to ensure adequate protein levels, the International Institute of Medicine recommends a dietary reference intake for pregnant women of 1.1 g/kg of body weight/day of protein (IOM, 2006). This is moderately higher than the standard 0.8 g/kg body weight/day recommended for non-pregnant adult women (IOM, 2006). Red meat, on average, contains 19-24 g of protein per 100 g (raw weight) while cooked red meat contains 27-35 g of protein per 100 g (cooked weight). Meat can be part of a healthy diet during pregnancy. Cooked red meat contains higher protein content due to the water content decreasing and nutrients become more concentrated during cooking (Williams, 2007). Protein from meats is highly digestible, around 94 % compared to the ileal digestibility of 78 % in beans and 86 % in whole wheat (Bhutta, 1999).

Pregnancy complicates the already complex metabolism of AA. Expansion of blood volume and growth of the maternal tissues require substantial amounts of protein (IOM, 1990). Growth of the fetus and placenta also places protein demands on the pregnant woman. Thus, additional protein is essential for the maintenance of a successful pregnancy. Maternal protein restriction, alone and in combination with energy restriction, results in consistently decreased fetal growth in many species (Rosso, 1977; Rosso and Streeter, 1979; Lederman and Rosso, 1980; Rosso, 1980; Pond et al, 1989).

There are dispensable and indispensable amino acids. The following AA are considered indispensable because they cannot be synthesized by mammals and must be obtained from dietary sources: phenylalanine, valine, threonine, tryptophan, isoleucine, methionine, histidine, arginine, leucine, and lysine. However, it should be recognized that all of the twenty protein AA

and their metabolites are required for normal cell physiology and function (Novelli and Tasker, 2007; El Idrissi, 2008; Lupi et al., 2008; Phang et al., 2008). Abnormal metabolism of any amino acid can disturb whole body homeostasis, impair growth and development, and may even cause death (Wu et al., 2004; Orlando et al., 2008; Willis et al., 2008).

Phenylalanine is an indispensable AA that is needed for normal functioning of the central nervous system. Isoleucine, leucine, and valine all work with one another to promote lean muscle-mass, normal growth, repair tissues, regulate blood sugar, and provide the body with energy (Bender, 2012). Branched-chain AA act as nitrogen carriers which assist the muscles in synthesizing other AA needed for anabolic muscle action. Additionally, branched-chain AA stimulate production of insulin which regulates circulating blood sugar to be taken up by the muscle cells and be used as energy. In addition to protein synthesis, threonine is an essential AA that promotes normal growth by helping to maintain the proper protein balance in the body. Threonine is also important for the formation of tooth enamel protein. Additionally, threonine is needed to create glycine and serine, two AA that are necessary for the production of collagen, elastin, mucin, and muscle tissue (Wu, 2009).

Tryptophan is a direct precursor for the neurotransmitter serotonin and melatonin (Slominski et al., 2002). Methionine is one of the essential sulfur-contain AA. Methionine is important for many bodily functions including 1-carbon metabolism, immune call production, and proper nerve function. Methionine is an essential AA important for normal closure of the neural tube. Insufficient methionine intake is suggested to increase the risk of neural tube defects in newborns (Dunlevy et al., 2006). Additionally, many studies in animals have shown that dietary supplements of AA, particularly lysine, increase calcium absorption and ultimately can play a critical role in bone health (Civitelli et al., 1992). Lysine is one of the primary components

of collagen and is essential for healthy collagen formation. Histidine is an essential AA that initially was thought to only be needed in the diets of children; however, experiments indicate that adults exposed to prolonged deficiencies of this AA have shown compromised health and bodily functions (Scogna, 2014). Histidine is a direct precursor of histamine (Voet and Voet, 1995), which possesses reparative properties when released upon tissue damage or in neutralization of antigens. Arginine is considered to be a semi-essential, or conditionally essential, AA meaning that it is required depending on the development stage or health status of an individual (Tapiero et al., 2002; Stipanuk and Caudill, 2013). Adult humans can synthesize arginine in sufficient amounts via the urea cycle. However, infants and young children are unable to effectively synthesize arginine, making it nutritionally essential for the maintenance of normal growth (Scogna, 2014) because it increases the production and release of somatotropin (Alba-Roth et al., 1988).

Iron

Adequate consumption of iron becomes particularly important during pregnancy. During pregnancy a woman's blood volume increases by nearly 50 % (ACOG, 2018a). This increased blood volume brings oxygen and nutrients to the woman's growing placenta and fetus. Without iron, the mother's blood would have less oxygen-carrying capacity and, since the body cannot produce iron endogenously, the iron content of the foods consumed is extremely important.

Iron is necessary for proper maternal nutrition as well as fetal and placental development. During pregnancy, iron is needed to expand the maternal red cell mass to facilitate oxygen delivery to the fetus and maternal muscles to help avoid muscle weakness and fatigue and reduce incidence of gestational irritability and depression (American Pregnancy Association, 2018b). Roughly, 19 % of pregnant women in the United States are diagnosed iron deficient with 7 % in

the first trimester and 30 % by the third trimester (Mei et al., 2011). According to the Academy of Nutrition and Dietetics, iron deficiency is the most common nutritional deficiency for pregnant women (Kominjarek and Rajan, 2016). It is recommended to increase iron consumption from 18 mg/day to 27 mg/day during pregnancy to prevent iron deficiency and anemia (NIH, 2018a). Red meats roughly contain about 3 mg of iron per 3 oz serving, which is significantly higher than other types of meat. For example, 3 oz of chicken, fish, or pork provide approximately 1 mg of iron. Beef and pork are the top food sources for bioavailable iron in the American diet, with beef having the highest content of heme iron (USDA-ARS, 2013).

There are two dietary iron compounds that are differentiated with respect to the mechanism of absorption; heme iron (derived from hemoglobin and myoglobin) and non-heme iron (derived mainly from cereals, fruits, and vegetables). Plant based foods are composed of non-heme iron. The iron content found in red meat is much more bioavailable than other food sources and is roughly 50 to 60 % heme iron (Higgs, 2000; Aggett, 2012). The absorption of these two dietary iron compounds is influenced differently by dietary factors (Hallberg, 1981). Factors influencing the absorption of iron from the diet are the amounts of heme and non-heme iron, the content of the dietary factors influencing iron bioavailability, and the iron status of the subjects (Lonnerdel, 2000; Abbaspour et al., 2014). As noted by Hurrell and Egli (2010), the bioavailability of iron is approximately 14 to 18 % from mixed diets, which include a considerable amount of red meat, seafood, and vitamin C (ascorbic acid, which enhances the bioavailability of non-heme iron) when compared to the 5 to 12 % from vegetarian diets. Red meat can also enhance the absorption of non-heme iron (Binnie et al., 2014) whereas phytate and certain polyphenols in non-animal proteins can inhibit iron absorption post-digestion (Murray-Kolbe and Beard, 2010).

Heme iron, although mostly consumed in smaller amounts, is 2 to 3 times more bio-available than non-heme iron and between 15 to 35 % is absorbed (Kalphalathika et al., 1991; Turhan et al., 2004). Heme iron is much less affected by other dietary factors (Carpenter and Mahoney, 1992; Clark et al., 1997; Yip, 2001; Turhan et al., 2004) and contributes significantly to absorbable iron. The total iron content of food needs to be further investigated and the heme- and non-heme fractions reported to facilitate development of a sustainable food-based approach to combat iron deficiency.

Calcium and Vitamin D

The high fetal demand for calcium in pregnancy is facilitated by profound physiological interactions between mother and fetus. The average consumption of calcium in western countries is about 800 mg, which does not meet the current recommendations for pregnant women (NIH, 2018b). The American College of Obstetricians and Gynecologists recommends 1,000 mg per day for pregnant and lactating (breastfeeding) women (ACOG, 2018b). Therefore, calcium consumption in pregnancy should be encouraged, especially during the second and third trimester of pregnancy and during lactation. Calcium is especially important for fetal skeletal development, primarily in the last trimester (American Pregnancy Association, 2018a).

Vitamin D is a cofactor with calcium for the development of fetal bones and teeth and is essential for healthy skin and eyesight (Wanger et al., 2012; Richer and Pizzimenti, 2013). Women, including those who are pregnant, need roughly 600 international units of vitamin D a day (Wanger et al., 2012). Vitamin D plays a role in maternal health including immune function, healthy cell division, and bone health and is necessary for the absorption and metabolism of calcium and phosphorus (Thorne-Lyman and Fawzi, 2012; Wanger et al., 2012). Some studies suggest a connection between low serum vitamin D levels and an increased risk of certain types

of cancers, autoimmune disease, neurological disease, insulin resistance, and cardiovascular disease (Martins et al., 2007; Pludowski et al., 2013; Autier et al., 2014). Animal based foodstuffs, including red meat, are the main sources of naturally occurring vitamin D (Williams et al., 2005; Schmid and Walther, 2013). Vitamin D deficiency is related to preeclampsia (Hollis et al., 2011). Preeclampsia is a pregnancy-specific syndrome that affects approximately 3-7 % of first pregnancies (Jeyabalan, 2013). The pathogenesis of preeclampsia involves a number of biological processes that may be directly or indirectly affected by vitamin D, including immune dysfunction, placental implantation, abnormal angiogenesis, excessive inflammation, and hypertension (Evans et al., 2004; Cardus et al., 2006).

Conclusion

There are two primary diagnoses of DM: Type I and Type II. It is important to note, however, that some women can develop a form of diabetes known as gestational diabetes when they become pregnant. This health problem for pregnant women has become more common in the past three decades and can potentially have adverse health outcomes, not only on the mother, but also on her offspring. Since these adverse health effects can be short- or long-term, it is important to understand the burden that gestational diabetes imposes on the population. Management of physical and nutritional health is vital to ensure the overall well-being of the mother and the growing fetus. It is important to ensure proper macro- and micronutrients are being consumed during pregnancy for the overall health of the mother and offspring. Ultimately, under- or over-nutrition can be associated with many adverse pregnancy outcomes to both the mother and offspring. Although pregnant women should steer clear of raw and undercooked meat, eating more red meat can help meet the body's new nutritional needs. It is important to

continue to examine the health effects and potential benefits provided by red meat during pregnancy.

Cholesterol and Monitoring Health Status

Introduction

Cholesterol is a type of fat that is part of all animal cells. Additionally, cholesterol is vital for overall health and is essential for many metabolic processes within the body. For example, the body uses cholesterol to build the structure of cell membranes; is a precursor to hormones such as estrogen, testosterone and adrenal hormones; produce bile acids which assist the digestion of fat and absorb important nutrients; and is an essential precursor for vitamin D production (Bridger, 2017). The human liver produces approximately 1-2 g of cholesterol per day (Sul and Storch, 2013). Roughly 80 % of cholesterol is synthesized by the liver and intestines; however, synthesis occurs in all nucleated cells including the skin, adrenal cortex, and gonads (Horton, 2002).

Measuring Cholesterol

There are no direct symptoms of high cholesterol; therefore, a blood test is necessary to determine the level of cholesterol within the blood stream. A complete cholesterol test (also called a lipid panel or lipoprotein profile) is a blood test that determines the concentration of cholesterol and triglycerides (**TG**) in an individual's blood (NIH, 2015). Ultimately, the determination of whether blood cholesterol levels are high may aid in estimating risk factors for developing heart disease and other comorbidities (Gordon et al., 1989; Liu et al., 2006; Yusuf et al., 2016). A lipoprotein profile is most commonly collected after a 9- to 12-h fast and a small sample of blood is taken from the finger or arm.

Factors Affecting Cholesterol Concentrations

Various factors can influence changes in circulating levels of cholesterol. Some factors can be modified and managed through general healthy lifestyle practices, while others cannot be changed. Heredity, age, and sex are examples of factors that cannot be controlled when managing cholesterol levels. High blood cholesterol can be hereditary, as genetics influence the amount of LDL produced and how fast it is removed from the system (Grundy et al., 2005). Blood cholesterol begins to rise around age 20 and continues to increase until age 60 or 65 (Félix-Redondo, 2013). Before age 50, male total cholesterol levels tend to be higher than females of the same age, while after age 50, the opposite occurs because female LDL levels often rise with menopause (NIH,2015). For children, the National Heart, Lung, and Blood Institute recommends one cholesterol screening test between the ages of 9 and 11 and another cholesterol screening test between the ages of 17 and 21 (NIH, 2015). Cholesterol testing is usually avoided between the ages of 12 and 16 because the hormones prevalent during puberty often contribute to false-negative results (Zachariah, 2000).

Excess weight tends to increase circulating LDL levels, raises TG, and lowers HDL. This condition is called atherogenic dyslipidemia and is commonly present in obese individuals (Krauss, 1998; NCPE, 2002; Grundy, 2004). The LDL particles associated with the metabolic syndrome and atherogenic dyslipidemia tend to be small and dense, ultimately allowing LDL particles to filter more readily into the arterial wall (Krauss and Dreon, 1995; Grundy, 2004). Data from the Third National Health and Nutrition Examination Survey showed that the age-adjusted prevalence of metabolic syndrome was 23.7 % (Ford et al., 2002). The most common component of the metabolic syndrome was obesity (39 %), followed immediately by low HDL levels (37 %; Gustafson et al., 2007). Specifically, a negative correlation of HDL with obesity

seems to be strongest with central obesity in women, which is characterized by intra-abdominal or visceral fat deposition (Poiliot et al., 1991;Poiliot et al., 1992). Previous studies have focused on the alteration of HDL catabolism in circulation following elevated TG. However, recent findings suggest that liver and fat tissue play a pivotal role in obesity-related low HDL (Wang and Peng, 2011).

The amount of cholesterol present in the diet and the concentration of cholesterol in blood are very different things. Although it may seem logical that eating cholesterol would raise blood cholesterol levels, it is now accepted that dietary intake of cholesterol has little bearing on plasma cholesterol (USDHHS and USDA, 2015). The body tightly regulates the amount of cholesterol produced and released into the blood. For example, when dietary intake of cholesterol goes down, the body makes more; inversely, when larger amounts of cholesterol are consumed, the body makes less (Goedeke and Fernandez, 2012). Because of this, foods high in dietary cholesterol have very little impact on blood cholesterol levels in most people (McNamara, 2000; Berger et al., 2015). However, about 25 % of the American population are classified as "hyper-responders." For these people, high-cholesterol foods do cause a rise in blood cholesterol. This condition is considered to be genetic (Weggemans et al., 2001; Kratz, 2005).

Fasting versus Non-Fasting Cholesterol

It has been common medical practice that individuals consume no food or liquids (other than water) for 9 to 12 h before a lipid profile test is performed. Rifai et al. (2016) summarized the rationale behind the fasting requirement: postprandial changes in lipoprotein, particularly with TG, are known to occur and these changes are directly related to the fat and carbohydrate content of the meal; an increase of TG (> 400 mg/dL) has a significant impact on the calculation

of LDL cholesterol when using the Friedewald equation; and treatment goals have been established from fasted sampling as the standard operating procedure for lipid measurement in previous clinical trials and epidemiological studies. Recent efforts have been made to simplify blood sampling methods by replacing fasting lipid profiles with non-fasting lipid profiles (Farukhi and Mora, 2016; Nordestgaar, 2017; Cartier et al., 2018). Some studies have found that lipids, lipoproteins, and apolipoproteins differ very little when comparing fasting to non-fasting with the exception of TG (Nordestgaard and Benn, 2009; Nigam, 2011). Additionally, evidence is lacking that fasting is superior to non-fasting when evaluating the lipid profile for cardiovascular risk assessment (Nigam, 2011; Van Dieren et al., 2011; Langsted and Nordestgaard, 2018).

In recent years, collecting a fasted sample to determine future risk of cardiovascular disease has been challenged because humans spend the majority of their waking moments predominately in the postprandial state (Rifai et al., 2016). Most individuals consume several meals during the day and some consume snacks between meals, resulting in the postprandial state predominating over a 24 h period (Saad et al., 2012). However, in clinical practice, the lipid profile is conventionally measured in blood plasma or serum obtained after fasting for at least 8 h and, therefore, may not reflect the daily average plasma lipid and lipoprotein concentrations and associated risk of cardiovascular disease (Rifai and Warnick, 2006; Simundic et al., 2014).

Swine and Cholesterol

Western societies continue to report high rates of cardiovascular disease even with advances in medicine and research. Research using human subjects is associated with substantial methodologic and ethical considerations and the quest for an appropriate animal model for the human cardiovascular system has led to swine (Lelovas et al., 2014.). The porcine heart has

many similarities to the human heart in terms of its coronary circulation and hemodynamic

similarities (Lelovas et al., 2014). Like humans, swine are omnivores. Because of this, their

vascular response to an increase in fat content in their diet is similar (Hamamdzic and Wilensky,

2013).

Swine have previously been used as a biomedical model in research for examining

atherosclerosis (Bloor et al., 1992; Gerrity et al., 2001; Suzuki et al., 2001; Natarajan et al., 2002;

Lelovas et al., 2014). As shown by Liedtke et al. (1975), swine have circulating LDL and HDL

cholesterol levels that are almost identical to that of humans (60 % and 38% vs. 63% and 28%,

respectively). Similar to humans, pigs can spontaneously develop atherosclerosis from aging

(Bloor et al., 1992; Lelovas et al., 2014). Feeding diets high in fat and cholesterol to commercial

swine to study diabetes mellitus resulted in accelerated atherosclerosis (Gerrity et al., 2001;

Suzuki et al., 2001; Natarajan et al., 2002). These swine developed hypercholesterolemia;

however, normal circulating concentrations of glucose and TG were observed (Gerrity et al.,

2001; Suzuki et al., 2001; Natarajan et al., 2002).

Conclusion

Cholesterol is a fatty substance essential to many processes within the body and it is

necessary for proper metabolic functions. There are two ways the body can obtain cholesterol,

the body can either make its own or cholesterol can be consumed from the diet. The liver is the

main processing center for cholesterol and dietary fat. Too much cholesterol circulating within

LDL in our bloodstream can lead to fatty deposits and arterial plaque developing in the arteries.

This may cause the vessels to narrow, block and lead to heart disease or stroke. While fasting

cholesterol measurement has long been the accepted convention, recent research may indicate

that non-fasting cholesterol measurements predict outcomes at least as well or better than fasting

cholesterol measurements. Swine have been, and continue to be used, as a biomedical model in research for examining the effects of cholesterol and health specifically regarding atherosclerosis.

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CHAPTER 3. THE EFFECTS OF RED MEAT OR SUGAR SUPPLEMENTATION DURING GESTATION AND LACTATION ON MATERNAL REPRODUCTION, MATERNAL AND OFFPSRING BLOOD PARAMETERS, AND OFFSPRING BODY COMPOSITION[1]

Abstract

Using swine as a model for humans, the objectives of this project were to determine if dietary supplementations of ground beef (**GB**), sugar (**SUG**) or a combination of the two (**SB**) during pregnancy would affect sow reproductive performance, early offspring growth, and sow risk factors for obesity-related metabolic disorders. Maternal type sows of at least 1 yr old at breeding were utilized for this study. Upon reaching D-40 of gestation, sows were randomly assigned to 1 of 4 dietary supplementation treatments: Control (**CON**): corn-soy gestation feed; GB: 4 oz cooked beef patty; SUG: sugar on a caloric equivalent to the beef patty; and SB: half of a 4 oz beef patty and a sugar mixture. The CON sows had the fewest piglets farrowed per litter compared to sows on GB, SUG, or SB treatments ($P < 0.04$), with no difference in total number farrowed between GB, SUG, and SB sows ($P > 0.6$). Although GB sows had piglets with a lower average birth weight than CON sows ($P = 0.02$), there was no difference in average piglet growth during lactation ($P = 0.17$) or in weaning weight ($P = 0.08$). There were no differences ($P >$

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[1] The material in this chapter was co-authored by A. M. Siomka, W. L. Keller, J. M. Young, and E. P. Berg. A. M. Siomka was responsible for feeding animals, bleeding and weighting animals, assisted in ultrasounds, assistance during farrowing, milk colocations, processing and weaning piglets, supervising undergraduate and other graduate students involved in project, coordination of events pertaining to slaughter, all laboratory work associated with project except feed analysis, and preparation and revision of this chapter. W. L. Keller was assisted in designing and running laboratory work. J. M. Young provided statistical guidance, assisted with bleeding and weighing, performed carcass ultrasounds, performed farrowing and weaning procedures, and assisted with proof reading. E. P. Berg was responsible study designed, assisted with slaughter and tissues collections, assisted with statistical analyses, and proofreading.

0.05) in traits measured on the sow (subcutaneous backfat depth, body weight, and energy balance). While there was no treatment effect seen in circulating low-density lipoprotein cholesterol (LDL; $P = 0.31$), serum concentrations of LDLch continued to decrease for SUG sows from early to late gestation. There were differences for high-density-lipoprotein cholesterol with GB sows having the greatest circulating levels compared to CON and SUG during mid gestation (HDL; $P = 0.05$ and 0.004, respectively). There were treatment differences seen during mid- and late gestation with GB sows having higher total cholesterol than SUG and CON sows ($P < 0.05$). Total cholesterol was higher for GB than SB during mid gestation ($P = 0.006$), resulting in slightly higher total cholesterol for GB during late gestation ($P = 0.08$). It can be concluded that the supplementations provided during gestation and lactation had a minimal effect on overall maternal reproduction and health and early offspring development and growth. When taking into consideration the size of the sows and the small percentage of the diet that came from the supplementation, a greater amount of added protein as beef or added sugar may have been needed to see more health altering effects. Additionally, this study showed that when ingesting beef or sugar on an equal caloric basis, adverse offspring development and growth was not present.

Introduction

The prevalence of gestational diabetes mellitus (**GDM**) is increasing worldwide. While the complications of GDM affecting the mother and fetus have been studied over the years, it is important to evaluate how the diet during pregnancy can impact these public health concerns. A modification to maternal diet offers potential to not only reduce adverse health outcomes for the mother but can also play an important role in the growth and development of the offspring. While red meat is a nutrient dense source of protein, zinc, and iron, all of which are essential

nutrients that pregnant woman should consume, it is often considered a negative food source as part of a Western diet (McNeil, 2014). When consuming a Western diet pattern, it is important to evaluate the components within the diet that may contribute to the increased public health concerns associated with GDM. The two components of a Western diet that are of greatest concern to consumers and health professionals are increased intake of sugar and red meat. This study will examine the effects of added sugar and added protein in the form of ground beef on maternal reproduction and health and offspring development.

Materials and Methods

This study was conducted at the North Dakota State University (**NDSU**) Swine Research Unit (Fargo, ND). All animal care and handling procedures were approved by the Institutional Animal Care and Use Committee (protocol #A17010) under the supervision of the attending veterinarian.

Animals

All animals used in this study were provided by the NDSU Swine Research Unit. Sows selected were of parity 2 or older. This was done to ensure that the sows had finished developmental growth. Additionally, this allowed for only those sows housed in individual stalls to be used. Gilts are pen-housed, so the confounding effects of housing type with treatment are avoided by using only parity 2 or older sows. Purebred Yorkshire sows and Chester White $\times$ Yorkshire sows were chosen for this study due to their high conception rates and maternal breed traits. This project was completed across 4 repetitions to achieve a total number of 35 sows; 8 sows in phase 1, 9 in phase 2, 10 in phase 3, and 8 in phase 4. Sows were bred via artificial insemination using 2 full-sibling boars to minimize genetic variation of the offspring. At day 30 (D- 30) of gestation, confirmation of pregnancy was confirmed by ultrasonography using a

portable animal ultrasound machine (Veterinary Ultrasound Scanner; Model 8300 Class I Type B, Power AC 100~240v, 3A, 50Hz). After confirmation of pregnancy, sows were moved into the gestational research room and placed into individual stalls. For environmental enrichment, sows were provided with nose-to-nose contact with neighboring sows and music. Additionally, chains were attached to the top of the stalls to allow sows another source of stimulation and would allow for chewing or shaking similar to a dog tugging on a rope or chew toy. Giving these "toys" helped prevent boredom and provided additional stimulation, which can help reduce excitability. Day-length was controlled by turning lights on at 0700 and off at 1900, allowing for a total of 12 h of light per day. All windows were covered and blacked out to ensure no natural light was entering from outside the gestational research room.

Feeding Protocol

Sows were fed a standard gestation diet (Table 3.1) following the guidelines of the National Research Council (**NRC**) dietary recommendations for swine (NRC, 1998) throughout the 4 repetitions of the study. All sows were provided *ad libitum* access to water. The basal gestation diet was formulated to meet the nutritional requirements for metabolic energy, amino acids, vitamins, and minerals. The ingredient composition and formulated nutrition content on an as-fed basis of the basal diet is presented in Tables 3.1 and 3.2, respectively. The basal gestation diet was fed at 0800 in the NDSU Swine Research Unit's production gestation room and at 0700 in the research gestation room. Sows were fed 2.04 kg of the basal gestation diet and adjusted as needed. Feed adjustments were made according to tissue ultrasound analysis performed every 2 wk. Ultrasonic determination of subcutaneous back fat was done to ensure that body condition (as designated by external fat thickness) was maintained within an optimal range to allow for the best reproductive performance. If the sow became too fat, the daily amount of the basal gestation

diet provided to that individual sow each morning was reduced. If the sow possessed a thin body composition with little to no subcutaneous fat backfat, the basal gestation diet would be increased to ensure proper nutrition and energy was being provided for both the sow and the growing fetuses. The dietary supplementation treatments were administered at 1100, 1500, and 1900 each day. Any feed not consumed by the sow was removed from the feeding trough and weighed before new feed was provided.

Table 3.1. Ingredient composition of the basal diet (as-fed basis).

Ingredients[1]	Gestation	Lactation
Corn	70.80	71.60
Soybean meal	9.85	23.35
Soybean hulls	15.00	0.00
Monocalcium	1.47	1.42
Limestone	1.06	1.10
Fat	0.75	1.25
EnMax Sow Premix 10[2]	0.50	0.50
Salt	0.45	0.45
Choline 60 (Dry)	0.12	0.12
L-Lysine	0.00	0.12
L-Threonine	0.00	0.06
DL-Methionine	0.00	0.03

[1] Ingredients are given on a percent basis of the total ration.
[2] Preformulated vitamin/mineral mix produced by Ralco Nutrition.

Table 3.2. Formulated nutrition content of the basal diet (as-fed basis).

Nutrient	Gestation	Lactation
Net energy, Mcal/kg	2.31	2.42
Crude protein, %	11.22	16.02
Lysine, %	0.70	1.10
Calcium, %	0.78	0.80
Phosphorus, %	0.60	0.65

Upon reaching D-40 of gestation, sows were randomly assigned to 1 of 4 supplementation treatments: beef (**GB**): consumption of basal gestation diet and a

supplementation of a 4 oz cooked beef patty (Food Service Direct, Hampton, VA); sugar (**SUG**): consumption of basal gestation diet and supplementation of sugar with the caloric equivalent to the beef patty; half sugar and half beef (**SB**): consumption of basal gestation diet and a supplementation of a beef and sugar mixture with the total combination being calorically equivalent to GB; and control (**CON**): consumption of basal gestation diet and a supplementation of the basal gestation diet at a caloric equivalent to the beef patty.

Body Composition and Blood Collections

Body weights were obtained every 2 wk during gestation starting at D-30 between 0800 and 0830 to monitor growth. An Aloka SSD-500V ultrasound machine fitted with a 3.5-MHz, 12.5-cm, linear array transducer (Corometrics Medical Systems, Inc., Wallingford, CT) operated by a trained ultrasound technician was used to obtain subcutaneous backfat depth (**BF**) adjacent the 10th and last thoracic ribs. This was done to ensure that BF was maintained within an optimal range to allow for the best reproductive performance. Additionally, sows were weighed and had ultrasonic BF measurements recorded at entrance to the farrowing room and at weaning.

Blood samples were collected prior to the start of dietary supplementation on D-39 of gestation and then subsequently on D-72 and D-100. Immediately after blood samples were obtained, a blood analysis was conducted using the iSTAT point of care device (Abaxis, Inc., Kansas City, MO). For iSTAT analysis, blood samples were collected in 3-mL lithium heparin tubes and CHEM8+ cartridges (Abaxis, Inc., Kansas City, MO) were used to measure concentrations of sodium (**Na**), potassium (**K**), chloride (**Cl**), ionized calcium (**iCa**), total carbon dioxide (**TCO$_2$**), glucose (**GLU**), blood urea nitrogen (**BUN**), creatinine, hematocrit (**Hct**), hemoglobin (**Hgb**), and Anion Gap (**AnGap**).

Separate blood samples for serum insulin and lipid panel determination were collected in 15-mL standard glass blood collection tubes fitted with silicone-coated stoppers. Blood was allowed to clot in a cooler on wet ice for up to 1 h, then centrifuged (swinging bucket TS-5.1-500, 3000 x *g*, 20 min, 4 °C; Allegra 25R Centrifuge, Beckman Coulter, Fullerton, CA). Serum was aliquoted, frozen, and stored at -20 °C until analysis of insulin and a full lipid panel [total cholesterol (**TOTch**), low-density lipoprotein cholesterol (**LDLch**), high-density lipoprotein cholesterol (**HDLch**), and triglycerides (**TG**)].

Farrowing and Lactation

Once sows reached their individual D-110 of gestation, they were weighed, washed, and moved into the farrowing room. Sows were housed individually in standardized farrowing stalls and fed 1.4 kg of a standard NRC lactation diet at 0700 and 1500 each day. Once sows farrowed, feed was gradually increased at each feeding to a maximum of 3.6 kg/204 kg body weight. Sows were fed to appetite to the point where they were able to empty the trough between feedings. Feed that was not consumed was weighed back as necessary. Feed consumption and any feed refusals were recorded daily. Supplementation was continued in lactation with supplements being provided at 1100, 1500, and 1900. All sows had *ad libitum* access to water.

All piglets were recorded as liveborn, dead at birth, stillborn, or mummy at farrowing. To distinguish between piglets dead at birth and stillborn, piglets were necropsied for lung tissue, which was evaluated for the presence of air. If air was present in the lungs (bright pink, fluffy lungs that floated in water), the piglet was identified as dead at birth. If air was not present in the lungs (dark reddish-purple, dense lungs that sank in water), the piglet was identified as stillborn. If present at farrowing, time born was recorded as well as presentation at birth: head first or breech (distinguished between posterior or rear feet first). A nontoxic semi-permanent marking

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crayon was used to record piglet number on the back. This number was utilized to later identify that piglet during neonatal processing. After farrowing was completed, NDSU Swine Research Unit's standard D-0 production processing was conducted, and farrowing date recorded. Sex, birth weight, and teat count were recorded for each piglet. Additional measurements taken during D-0 processing included crown-rump length, abdominal girth, and chest girth on all piglets. Piglets were ear notched for identification and needle teeth clipped. On D-2, NDSU Swine Research Unit's standard D-2 processing procedures were conducted with one exception; male piglets were not castrated. Piglets had their tails docked and were given 1 cc each of iron (Uniferon 200, Pharmacosmos, Inc., Watchung, NJ) and Duramycin 72-200 (Durvet, Inc., Blue Springs, MO) intramuscularly on opposite sides of the neck. Because this project was biomedical in nature, male piglets were not castrated at this time. In addition to birth weight, piglet body weights were recorded on D-6, D-12, and D-18 (weaning). For any piglets that died during the suckling period, date of death and weight at death were recorded. At weaning, the median male and female from each litter were determined. The selected male was kept intact, and all other males were castrated utilizing a lidocaine (Lidocaine HCl 2% Injection, MWI Animal Health, Boise, ID) block prior to castration. The selected male and female were transported to the NDSU Animal Nutrition and Physiology Center (Fargo, ND) for further research not presented in this chapter.

After farrowing and during lactation, milk samples were collected from each sow at D-0, D-6, D-12, and D-18 of lactation. Milk samples were obtained by hand-milking 2 to 4 teats after intramuscular injection of 1 cc of oxytocin (Oxytocin Injection, Agri Laboratories Ltd., St. Joseph, MO). Hand milking began after the birth of the first piglet (range of 0-4 h), by which additional piglets have been born but no placenta has been passed. Milk was obtained using a

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stripping action from the front 2 teats on the left and right sides of the sow to obtain a total of 30 mL per sample. The milk for each sow was collected in a screw-top sample jar and then transferred to breastmilk storage bags (Lansinoh, Alexandria, VA) and kept frozen at -20 °C until ready for milk composition analysis of percentage of fat, protein, lactose, total solids, other solids, and milk urea nitrogen (**MUN**).

Piglet Traits

As the experimental unit for this research project was the sow, traits measured on the piglets were evaluated as a trait of the sow. Piglet traits were considered to be total number farrowed, number fully formed, number born alive, number dead at birth, number of stillborn, number of mummies, farrowing survival, sex ratio, total litter birth weight, total live piglet birth weight, average piglet birth weight, average live piglet birth weight, average crown-rump length, average abdominal girth, average chest girth, number of piglets on D-6, total litter weight on D-6, average piglet weight on D-6, number of piglets on D-12, total litter weight on D-12, average piglet weight on D-12, number of piglets on D-18, pre-weaning survival, total litter weight on D-18, average piglet weight on D-18, litter average daily gain, litter growth, litter energy gain, average piglet average daily gain, average piglet growth, and average piglet energy gain.

Number fully formed was calculated as total number farrowed minus number of mummies. Farrowing survival was calculated as number born alive as a percent of total number farrowed. Sex ratio was calculated as the number of gilts divided by the number of boars in a litter. Total litter and average piglet birth weights were the sum and mean, respectively, of the birth weights of all fully formed piglets. Pre-weaning survival was the number of piglets on D-18 expressed as a percentage of the number born alive. Piglet growth was defined as D-18 weight (or weight at death) minus birth weight and was totaled (litter growth) and averaged (average

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piglet growth) by sow. Piglet average daily gain was calculated as piglet growth divided by 18 (or age at death). Piglet energy gain was estimated according to Bergsma et al. (2009) and Young et al. (2016).

Sow Traits

Sow traits were considered to be sow weight at farrowing, sow fat mass at farrowing, sow protein mass at farrowing, sow BF at farrowing, sow weight at weaning, sow fat mass at weaning, sow protein mass at weaning, sow BF at weaning, sow weight loss, sow fat mass loss, sow protein mass loss, sow BF loss, sow maintenance, sow total feed intake, energy balance, and lactation efficiency.

The sow body weight taken at entrance to the farrowing room was adjusted for estimated weight of piglets, placentas, and intrauterine fluid according to Noblet et al. (1985), Bersgma et al. (2009), and Young et al. (2016) to calculate sow weight at farrowing. To adjust for the change in water composition of the mammary glands, sow weight at weaning was calculated from the sow body weight recorded at weaning utilizing equations from Bergsma et al. (2009) and Young et al. (2016). Sow weight loss, fat mass loss, protein mass loss, and BF loss were calculated as the value at farrowing minus the value at weaning. As a result, a more positive value indicates a greater loss. Sow maintenance was estimated according to Bergsma et al. (2009) and Young et al. (2016) as the average maintenance requirement during lactation. Sow total feed intake was the sum of all feed offered during lactation minus any orts during lactation. Energy balance was defined as the difference between energy retained by the sow at weaning and energy retained by the sow at farrowing, with energy retained values being calculated according to Bergsma et al. (2009) and Young et al. (2016). Lactation efficiency was defined as the ratio of energy output (in the form of piglet growth and maintenance) to energy input (energy from feed and body tissue

mobilization above maintenance requirements of the sow) and was calculated according to

Bergsma et al. (2008, 2009) and Young et al. (2016).

Lipid Panel and Insulin Assays

Serum TOTch was determined using the Infinity Cholesterol Liquid Stable Reagent (TR 13421; Thermo Fisher Scientific, Inc., Middletown, VA) and 200 mg/dL Stock Cholesterol Standard (C7509-STD, Pointe Scientific, Inc.). The assay was modified for a microtiter plate reader and used a sample-to-reagent ration of 1:50, with a total reagent volume of 250 µL. Serum samples were incubated at 37 °C with a 10-min incubation time. Enzymatic (cholesterol esterase and cholesterol oxidase) colorimetric endpoint assay was read at a wavelength of 500 nm.

Serum LDLch was determined using the Liquid autoLDL Cholesterol Reagent Set [Reagent 1 and Reagent 2] (H7574-80; Pointe Scientific, Inc., Canton, MI) and 104 mg/dL autoHDL/LDL Cholesterol Calibrator (H7545-CAL; Pointe Scientific, Inc., Canton, MI). The assay was modified for a microtiter plate reader and used a sample-to-reagent 1 ratio of 1:45 and sample-to-reagent 2 ratio of 1:15, with a total reagent volume of 300 µL with a total sample-to-total reagent ratio of 1:60. Serum samples were incubated at 37 °C in Reagent 1 for 10 min. After the first incubation, Reagent 2 was added, and the sample was incubated for an additional 10 min at 37 °C. Colorimetric endpoint assay was read at a wavelength of 546 nm. Reagent 1 solubilizes only the non-LDLch particles. The cholesterol released is consumed by cholesterol esterase and cholesterol oxidase in a non-color forming reaction. Reagent 2 solubilizes the remaining LDLch particles and chromogenic coupler allows for color formation. The enzyme reaction with dihydrolipoamide dehydrogenase in the presence of the coupler produces color that is proportional to the amount of LDLch present in the samples.

Serum HDLch was determined using the Liquid autoHDL Cholesterol Reagent Set [Reagent 1 and Reagent 2] (H7545-80; Pointe Scientific, Inc., Canton MI) and 56 mg/dL auto HDL/LDL Cholesterol Calibrator (H7545-CAL; Pointe Scientific, Inc., Canton, MI). The assay was modified for a microtiter plate reader and used a sample-to-Reagent 1 ratio of 1:45 and sample-to-Reagent 2 ration of 1:15, with a total reagent volume of 300 µL with a total sample-to-total reagent ratio of 1:60. Serum samples were incubated at 37 °C in Reagent 1 for 10 min. After the first incubation, Reagent 2 was added, and the sample was incubated for an additional 10 min at 37 °C. Colorimetric endpoint assay was read at a wavelength of 600 nm. Reagent 1 contains α-cyclodextrin and dextran sulfate to stabilize LDLch, very low-density lipoprotein (VDLch), and chylomicrons. Reagent 2 contains PEG-modified enzymes that selectively react with the cholesterol present in the HDLch particles. Consequently, only the HDLch is subject to cholesterol measurement.

Serum TG were determined using the Infinity Triglyceride Liquid Stable Reagent (TR22421; Thermo Fisher Scientific, Inc., Middletown, VA) and 200 mg/dL Glycerol Standard Solution (T7531-STD; Pointe Scientific, Inc., Canton MI). The assay was modified for a microtiter plate reader and used a sample-to-reagent ration of 1:50, with a total reagent volume of 250 µL. Serum samples were incubated at 37 °C with an 8-min incubation at 37 °C. Colorimetric endpoint assay was read at a wavelength of 500 nm.

Serum insulin was determined using the Abclonal Porcine Insulin ELISA Kit (PI0011; ABclonal, Woburn MA). The plate was pre-coated with an insulin-specific antibody. Standard and the samples were co-incubated in the wells along with an insulin horseradish peroxidase (**INS-HRP**) conjugate. The insulin in the standards and in the samples compete with the INS-HRP conjugate for binding to the plate-bound antibody. Higher levels of insulin for the standard

or samples leads to a decrease in the INS-HRP conjugate binding and thus a reduced signal. The assay was a competitive immunoassay, was designed for a microtiter plate reader, and was run in triplicates. The colorimetric endpoint assay was read immediately at a wavelength of 450 nm.

Statistical Analysis

Analyses were conducted using the mixed procedure in SAS (v. 9.4, SAS Institute, Cary, NC). The model included fixed effects of treatment, repetition, and day (for traits occurring over time) and covariates of number piglet fully formed, and number born alive. All two- and three-way interactions between fixed effects were tested and removed from the model if $P > 0.10$ except for treatment by day interaction, which was left in the model regardless of P-value. For traits occurring over time, a repeated measures statement was used with sow as the subject. Different covariate methods for the repeated measures statement were tested and the best fit method based on AICC was chosen. Least square means were calculated.

Results

Two sows were removed from the study in the second repetition. One sow had a spontaneous abortion prior to the D-72 bleed. Her iCa was noticeably out of range (0.89 versus the 1.10-1.40 of all other values). She was ultrasounded for confirmation of pregnancy and had lost her pregnancy. The second sow became spraddle-legged (rear legs splayed laterally, unable to bear weight) around D-95 of gestation. She was allowed to farrow, but piglets were cross-fostered onto another sow immediately at birth because she was unable to turn over to nurse them. Although both sows removed from the trial were from the SB treatment, the researchers do not feel that the spontaneous abortion or the occurrence of the spraddle-leg condition was due to the effect of treatment.

Piglet Traits

Simple statistics for piglet traits are presented in Table 3.3 and least square means by dietary supplement treatment are presented in Table 3.4. The CON sows had the least number of total piglets farrowed per litter compared to sows on GB, SUG, or SB treatments ($P < 0.04$), with no difference in total number farrowed between GB, SUG, and SB sows ($P > 0.6$). However, farrowing survival rate was greatest for CON sows, with CON sows showing a tendency for a greater farrowing survival rate than SUG sows (96.6 vs. 88.3 %; $P = 0.09$) and GB and SB sows being intermediate. No significant differences were observed for number born alive; however, CON sows had an average of 2 fewer piglets born alive than GB, SUG, or SB sows ($P = 0.06$, 0.11, and 0.05, respectively). Because there was no significant difference in number of mummies ($P > 0.10$), CON sows had significantly fewer fully formed piglets than SUG or SB sows ($P < 0.04$) and tended to have fewer fully formed piglets than GB sows ($P = 0.05$).

There were no differences seen across treatments for number of stillborn piglets per litter; however, SUG sows had significantly more piglets dead at birth compared to GB sows ($P = 0.03$) and tended to have more piglets dead at birth than CON sows ($P = 0.07$). It is important to note that the rate of dead at births, stillborn, and mummies was low in this project, with all least square means for these traits being less than 1. Only SUG sows had a value for dead at birth significantly different from 0 ($P = 0.003$). Number of dead at birth tended to be different from 0 for CON sows ($P = 0.07$). Both GB and SUG sows had a number of stillborn piglets different from 0 ($P < 0.04$). Sows on the GB treatment were the only ones which had a number of mummies significantly different from 0 ($P = 0.03$).

In addition to pre-weaning survival percentage being lower in GB sows when compared with CON (81.5 vs. 96.5 %; $P = 0.04$), GB sows had lower piglet birth weights, average piglet

weights on D-6 (2.4 vs. 2.9 kg; $P = 0.009$), lower litter weights on D-6 (21.49 vs. 24.71 kg; $P = 0.05$) and lower average piglet weights on D-12 (3.7 vs. 4.5 kg; $P = 0.01$) when compared with CON sows. Lastly, overall live piglet birth weights were also higher for CON vs. SUG (1.8 vs. 1.6 kg; $P = 0.02$).

Table 3.3. Means, standard deviation (SD), minimum (Min), and maximum (Max) range for piglet traits per sow unit.

Trait	N[1]	Mean	SD	Min	Max
Total number farrowed, no.	34	10.62	2.79	4	16
Number fully formed, no.	34	10.47	2.70	4	15
Number born alive, no.	34	9.71	2.47	4	14
Number dead at birth, no.	34	0.29	0.63	0	2
Number of stillborns, no.	34	0.47	0.71	0	2
Number of mummies, no.	34	0.15	0.36	0	1
Farrowing survival, %	34	92.25	9.31	66.67	100.00
Sex ratio, no. gilts/no. boars	34	1.09	1.07	0.00	6.00
Litter birth weight, kg	34	17.11	4.23	8.66	26.58
Average litter birth weight, kg	34	1.66	0.23	1.08	2.35
Total live piglet birth weight, kg	34	16.02	3.96	6.98	22.90
Average live piglet birth weight, kg	34	1.67	0.22	1.16	2.35
Average piglet crown-rump length, cm	25	28.41	1.63	26.08	31.44
Average piglet abdominal girth, cm	24	23.32	1.70	20.88	26.93
Average piglet chest girth, cm	24	26.07	1.30	23.44	28.64
Number of piglets on D-6, no.	34	8.85	2.24	4	13
Total litter weight on D-6, kg	34	22.45	5.98	8.12	36.01
Average piglet weight on D-6, kg	34	2.57	0.44	1.62	4.06
Number of piglets on D-12, no.	34	8.47	2.78	0	13
Total litter weight on D-12, kg	33	35.08	9.30	8.93	52.65
Average piglet weight on D-12, no.	33	4.07	0.67	2.23	5.80
Number of piglets on D-18, no.	34	8.29	2.84	0	13
Total litter weight on D-18, kg	33	48.56	13.47	9.89	74.15
Pre-weaning survival, %	34	85.11	21.06	0.00	100.00
Litter average daily gain, g/d	33	1877.5	542.4	370.4	2910.1
Litter growth, kg	33	33.80	9.76	6.67	52.38
Litter energy gain, MJ ME/d	33	29.59	8.31	5.92	45.48
Average piglet average daily gain, g/d	33	224.0	40.2	144.2	309.3
Average piglet growth, kg	33	4.03	0.72	2.60	5.57
Average piglet energy gain, MJ ME/d	33	3.53	0.57	2.44	4.86

[1]With sow serving as the experimental unit, traits measured on the piglets were evaluated as a trait of the sow and therefore N = number of sows per trait.

Table 3.4. Least square means by treatment for piglet traits.

Trait	LS Means				SED[1]
	GB	SUG	SB	CON	
Total number farrowed, no.	11.2ᵃ	11.3ᵃ	11.9ᵃ	8.4ᵇ	1.4
Number fully formed piglets	11.0ᵃᵇ	11.2ᵃ	11.5ᵃ	8.4ᵇ	1.4
Number born alive	10.3ᵃ	10.0ᵃᶜ	10.6ᵇᶜ	8.0ᵃᶜ	1.3
Number dead at birth	-0.01ᵃ	0.69ᵇ	0.47ᵃ	0.13ᵇ	0.33
Number stillborn	0.66	0.57	0.46	0.25	0.41
Number of mummies	0.20	0.11	0.33	-0.00	0.19
Farrowing survival, %	92.0	88.3	90.8	96.6	5.2
Sex ratio, no. gilt/no. boars	0.84	1.30	1.42	0.96	0.61
Litter birth weight, adjusted for no. fully formed, kg	16.5ᵃ	16.8ᵇ	17.5ᵃ	18.3ᵃ	1.1
Average litter birth weight, adjusted for no. fully formed, kg	1.6	1.6	1.7	1.8	0.1
Total live piglet birth weight, adjusted for no. born alive, kg	15.5	15.8	16.2	17.1	0.9
Average live piglet birth weight, kg	1.7ᵃ	1.6ᵇᶜ	1.7ᵃ	1.9ᵃᶜ	0.1
Average crown to tail head length, cm	28.13	27.9	26.4	28.9	0.9
Average pig abdominal girth, cm	23.5	23.4	23.3	23.5	0.9
Average pig chest girth, cm	26.3	25.9	26.1	26.7	0.8
Number of piglets on D-6	9.2	9.1	9.6	7.8ᵇ	1.2
Total litter weight on D-6, adjusted for no. of piglets on D-6, kg	21.5ᵃ	22.5ᵃᵇ	22.3ᵃᵇ	24.7	1.7
Average piglet weight on D-6, kg	2.4ᵃ	2.6ᵃ	2.6ᵃ	2.9ᵇ	0.2
Number of piglets on D-12, no.	9.1	8.8	9.3	7.7	1.3
Total litter weight on D-12, adjusted for no. of piglets on D-12, kg	33.6	34.7	35.1	37.6	2.5
Average piglet weight on D-12, kg	3.7ᵃ	4.0ᵃ	4.2ᵃ	4.5ᵇ	0.3
Number of piglets on D-18	8.6	7.8	9.3	8.6	1.4
Total litter weight on D-18, adjusted for no. of piglets on D-18, kg	47.3	49.1	47.8	50.6	3.4
Average piglet weight on D-18, kg	5.4	5.8	5.7	6.2	0.4
Pre-weaning survival, %	81.5ᵃ	86.25ᵃ	90.0ᵃ	96.5ᵇ	7.7
Litter average daily gain, g/d	1839.6	1933.4	1856.4	1904.7	177.9
Litter growth, adjusted for no. piglets on D-18, kg	33.1	34.8	33.4	34.3	3.1
Litter energy gain, adjusted for no. piglets on D-18, MJ ME	29.0	30.3	29.3	30.1	2.4
Average piglet average daily gain, g/d	210.1	231.9	222.01	238.2	21.4
Average piglet growth, kg	3.8	4.2	3.9	4.3	0.4
Average piglet energy gain, MJ ME	3.3	3.6	3.5	3.8	0.3

[1]SED = greatest standard error of difference between treatments.
ᵃ,ᵇ Least square means differ by $P < 0.05$.

Sow Traits

Simple statistics for sow traits are presented in Table 3.5 and least square means by treatment are presented in Table 3.6. There were no significant differences seen in any of the sow traits ($P > 0.1$). However, there was a numeric advantage in lactation efficiency for GB sows compared to SUG sows ($P = 0.11$).

Table 3.5. Means, standard deviation (SD), minimum (Min), and maximum (Max) range for sow traits.

Trait	N	Mean	SD	Min	Max
Sow weight at farrowing, kg	26	245.7	32.0	186.1	295.3
Sow fat mass at farrowing, kg	26	48.3	14.1	23.4	81.6
Sow protein mass at farrowing, kg	26	38.3	4.7	26.7	44.0
Sow backfat depth at farrowing, mm	33	19.1	6.5	6.5	33.0
Sow weight at weaning, kg	30	256.7	31.5	190.0	311.1
Sow fat mass at weaning, kg	22	51.8	12.8	26.0	72.2
Sow protein mass at weaning, kg	22	40.0	5.0	27.6	48.9
Sow backfat depth at weaning, cm	24	19.0	5.5	9.5	27.5
Sow weight loss, kg	26	7.8	16.7	-46.8	27.2
Sow fat mass loss, kg	18	-0.59	9.07	-22.28	19.62
Sow protein mass loss, kg	18	0.76	2.77	-7.39	3.49
Sow backfat loss, mm	24	-1.46	4.37	-11.00	9.00
Sow maintenance, MJ ME/d	26	27.6	2.6	22.5	31.8
Sow total feed intake, kg	33	112.7	22.8	66.3	149.6
Energy balance, MJ ME/d	18	-0.30	20.88	-47.32	44.82
Lactation efficiency, %	18	3.17	1.82	0.92	7.98

Blood Chemistry

iSTAT

Simple statistics for blood chemistry traits are presented in Table 3.7 and least square means by treatment and day are presented in Table 3.8. Blood chemistry is often evaluated in human medicine as a means to screen for overall wellness and health status at a given point in time. Of the 11 blood parameters evaluated in the present study, BUN, TCO_2, Hct, and Hgb were the only traits significantly impacted by treatment.

Table 3.6. Least square means by treatment for sow traits.

Trait	GB	SUG	SB	CON	SED[1]
	LS Means				
Sow weight at farrowing, kg	260.2	262.4	276.8	283.2	18.4
Sow fat mass at farrowing, adjusted for weight at farrowing, kg	48.9	51.7	52.5	51.2	6.4
Sow protein mass at farrowing, adjusted for weight at farrowing, kg	38.2	37.7	37.6	37.8	1.0
Sow backfat depth at farrowing, mm	18.6	18.3	20.9	19.7	3.0
Sow weight at weaning, kg	254.1	255.1	253.9	265.9	19.7
Sow fat mass at weaning, adjusted for weight at weaning, kg	46.9	54.5	51.4	53.6	10.4
Sow protein mass at weaning, adjusted for weight at weaning, kg	39.8	39.8	38.2	42.6	3.9
Sow backfat depth at weaning, cm	16.7	19.8	19.9	19.4	3.9
Sow weight loss, adjusted for weight at farrowing, kg	6.36	10.11	2.90	1.40	10.52
Sow fat mass loss, adjusted for fat mass at farrowing, kg	-3.61	-3.25	-8.53	-1.55	6.21
Sow protein mass loss, adjusted for protein mass at farrowing, kg	1.06	1.92	0.34	0.25	2.54
Sow backfat loss, adjusted for sow backfat depth at farrowing, cm	-2.89	-1.21	-2.13	-1.13	2.17
Sow maintenance, MJ ME/d	27.1	27.4	27.8	28.1	1.8
Sow total feed intake, kg	108.1	120.0	118.8	104.4	12.4
Energy balance, MJ ME/d	-5.85	-5.11	-18.82	-3.88	14.48
Lactation efficiency, %	4.72	2.55	3.74	3.57	1.50

[1]SED = greatest standard error of difference between treatments.

Table 3.7. Means, standard deviation (SD), minimum (Min), and maximum (Max) range for iSTAT blood chemistry traits.

Trait	N	Mean	SD	Min	Max
Sodium	91	142.0	1.9	135.0	148.0
Potassium	91	4.16	0.40	3.40	5.50
Chloride	90	100.5	2.4	88.0	106.0
Ionized calcium	89	1.25	0.07	1.05	1.40
Total carbon dioxide	91	28.1	3.0	8.0	35.0
Glucose	91	71.4	7.2	53.0	91.0
Blood urea nitrogen	91	4.99	2.42	3.00	14.00
Creatinine	91	2.55	.50	1.30	4.00
Hematocrit	91	36.6	4.8	27.0	62.0
Hemoglobin	91	12.4	1.6	9.2	21.1
Anion gap	90	18.5	2.4	14.0	33.0

Table 3.8. Least square means (standard error) of blood chemistry components by treatment (TRT) and day of gestation (D-39, D-72, and D-100) for sows supplemented with beef (GB), sugar (SUG), combination of beef and sugar (SB), and a corn-soy control (CON) throughout gestation and lactation.

Traits[1]	D-39				D-72				D-100				P-values		
	GB	SUG	SB	CON	GB	SUG	SB	CON	GB	SUG	SB	CON	TRT	Day	TRT x day
Na,	142.1	141.3	142.3	141.7	141.3	142.4	141.9	142.1	142.2	142.5	142.1	141.5	0.90	0.82	0.63
mmol/L	(0.5)	(0.6)	(0.6)	(0.6)	(0.7)	(0.6)	(0.7)	(0.7)	(0.5)	(0.6)	(0.6)	(0.6)			
K,	4.21	4.09	4.15	4.17	4.17	4.24	4.16	4.18	4.04	4.30	4.27	4.11	0.88	0.93	0.72
mmol/L	(0.11)	(0.11)	(0.12)	(0.12)	(0.14)	(0.13)	(0.14)	(0.13)	(0.11)	(0.11)	(0.12)	(0.12)			
Cl,	100.4[ab]	99.4[a]	100.3[ab]	100.3[ab]	99.6[ab]	101.2[ab]	100.4[ab]	101.7[b]	100.5[ab]	100.6[ab]	100.7[ab]	101.8[b]	0.14	0.75	0.30
mmol/L	(0.7)	(0.7)	(0.7)	(0.7)	(0.9)	(0.8)	(0.9)	(0.9)	(0.7)	(0.7)	(0.8)	(0.8)			
iCa,	1.25[abc]	1.24[abc]	1.28[bc]	1.23[abc]	1.28[abc]	1.30[c]	1.28[abc]	1.26[abc]	1.24[abc]	1.23[ab]	1.26[abc]	1.22[a]	0.08	0.08	0.96
mmol/L	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.03)	(0.01)	(0.02)	(0.02)	(0.02)			
tCO$_2$,	28.1[abc]	27.3[abc]	28.5[bc]	28.0[abc]	28.2[abc]	29.6[c]	28.1[abc]	25.9[a]	28.0[abc]	29.5[c]	27.7[abc]	27.1[ab]	<0.01	0.98	0.30
mmol/L	(0.7)	(0.7)	(0.8)	(0.8)	(0.9)	(0.9)	(0.9)	(0.9)	(0.7)	(0.7)	(0.8)	(0.8)			
Glu,	69.3[a]	70.4[ab]	69.0[a]	70.6[ab]	71.2[ab]	71.8[ab]	73.3[ab]	77.0[b]	74.1[ab]	71.2[ab]	71.0[ab]	71.8[ab]	0.70	0.12	0.76
mg/dL	(2.1)	(2.1)	(2.3)	(2.3)	(2.7)	(2.5)	(2.7)	(2.7)	(2.2)	(2.1)	(0.5)	(2.4)			
BUN,	5.56[cde]	5.00[bcd]	5.60[de]	5.33[cde]	7.58[f]	2.90[a]	4.20[abc]	4.83[bcd]	6.80[ef]	3.70[ab]	3.54[ab]	4.71[bcd]	<0.01	0.17	<0.01
mg/dL	(0.55)	(0.55)	(0.58)	(0.58)	(0.65)	(0.62)	(0.66)	(0.66)	(0.55)	(0.55)	(0.61)	(0.60)			
Crea,	2.35[b]	2.35[ab]	2.35[ab]	2.36[ab]	2.64[acd]	2.48[abc]	2.40[ab]	2.46[ab]	2.80[d]	2.85[d]	2.80[d]	2.75[cd]	0.89	<0.01	0.72
mg/dL	(0.10)	(0.10)	(0.11)	(0.10)	(0.12)	(0.11)	(0.12)	(0.12)	(0.10)	(0.10)	(0.11)	(0.11)			
Hct,	36.3[abcd]	38.7[cde]	37.0[bcde]	36.6[abcd]	38.4[bcde]	34.5[ab]	39.4[de]	40.6[e]	35.5[abc]	33.2[a]	34.9[ab]	36.0[abcd]	0.24	<0.01	0.02
%PCV	(1.2)	(1.2)	(1.2)	(1.2)	(1.5)	(1.4)	(1.5)	(1.5)	(1.2)	(1.2)	(1.3)	(1.3)			
Hgb, g/dL	12.4[abcd]	13.2[cde]	12.6[bcde]	12.4[abcd]	13.1[bcde]	11.7[ab]	13.4[de]	13.8[e]	12.1[abc]	11.3[a]	11.9[ab]	12.2[abcd]	0.25	<0.01	0.02
	(0.4)	(0.4)	(0.4)	(0.4)	(0.5)	(0.5)	(0.5)	(0.5)	(0.4)	(0.4)	(0.4)	(0.4)			
AnGap,	18.4[abc]	19.5[c]	18.7[abc]	18.6[abc]	18.9[abc]	17.4[a]	18.8[abc]	19.6[bc]	18.9[abc]	17.8[abc]	18.7[abc]	17.4[ab]	0.60	0.55	0.20
mmol/L	(0.6)	(0.6)	(0.7)	(0.7)	(0.8)	(0.8)	(0.8)	(0.8)	(0.6)	(0.6)	(0.7)	(0.7)			

[1]Definitions: sodium (Na), potassium (K), ionized calcium (iCa), total carbon dioxide (tCO$_2$), glucose (Glu), blood urea nitrogen (BUN), creatinine (Crea), hematocrit (Hct), hemoglobin (Hgb), anion gap (AnGap).

[a,b] Least square means with different superscripts differ by $P < 0.05$.

For Cl, GB tended to be lower than CON ($P = 0.09$) on D-72 and SUG tended to be lower on D-39 than D-72 ($P = 0.09$). For iCa, there was a tendency for a treatment ($P = 0.08$) and a day ($P = 0.08$) effect. Sows on the CON treatment had a lower iCa than sows on the SB treatment (1.24 vs. 1.27 mmol/L, respectively; $P = 0.01$), while sows on the GB and SUG treatments were intermediate to CON and SB but not different ($P > 0.10$). While there was an overall decrease in iCa from D-72 to D-100 (1.28 to 1.24 mmol/L, respectively; $P = 0.03$), only SUG sows had a significant difference between D-72 and D-100 ($P = 0.046$).

For TCO_2, there was a significant treatment effect ($P = 0.003$). Sows on the CON treatment had lower TCO_2 values than sows on the GB ($P = 0.02$), SB ($P = 0.02$), and SUG ($P = 0.0002$) treatments. Sows on the GB and SB treatments did not differ in TCO_2 values ($P = 0.98$) and tended to have lower TCO_2 values than sows on the SUG treatment ($P = 0.09$). Although the treatment by day interaction was not significant, there were some differences between treatments across time. Sows on the CON treatment had lower TCO_2 than sows on the GB ($P = 0.09$), SUG ($P = 0.004$), and SB ($P = 0.09$) treatments on D-72. On D100, SUG was greater than CON ($P = 0.03$) and SB ($P = 0.09$). From D-39 to D-72, SUG saw an increase in TCO_2 ($P = 0.06$) and D-39 tended to be lower than D-100 ($P = 0.09$). For GLU, CON tended to be higher on D 72 than D 39 ($P = 0.07$), which is most likely the reason for a significantly higher GLU concentration on D-72 compared to D-39 across treatments (73.3 vs. 69.8 mg/dL, respectively; $P = 0.048$).

There was a significant ($P = 0.0005$) treatment by day interaction for BUN. It appears that the interaction of treatment and day is driven by differences in BUN during mid- and late gestation (D-72 and D-100) because there is no difference between treatments in early gestation (D-39; $P > 0.45$). The level of BUN was higher for GB sows on D-72 and D-100 than SUG ($P < 0.0001$ and 0.0002, respectively), SB ($P = 0.0005$ and 0.0002, respectively), and CON ($P = 0.004$

and 0.01, respectively). On D-72, CON sows had higher BUN levels than SUG sows ($P = 0.03$).

For GB sows, BUN increased from D-39 to D-72 ($P = 0.001$). While there was a numeric

decrease from D-72 to D-100 for GB sows, this was not significant ($P = 0.18$). However, D-100

BUN levels for GB sows tended to be higher than D-39 BUN levels ($P = 0.097$). For SB sows,

BUN levels decreased from D-39 to D-72 ($P = 0.03$) and again from D-72 to D-100 ($P = 0.32$),

resulting in a significant difference between D-39 and D-100 ($P = 0.01$). For SUG sows, BUN

levels decreased from D-39 to D-72 ($P = 0.0005$). There was a numeric increase from D-72 to D-

100 ($P = 0.16$), resulting in a tendency for D-100 BUN levels to be lower than D-39 BUN levels

($P = 0.09$) for SUG sows.

Creatinine levels increased across time for all treatments, although significance was

different from D-39 to D-72 and D-72 to D-100 for each treatment but all treatments had D-39

and D-100 values that were significantly different. Average creatinine levels were 2.35 on D-39,

2.50 on D-72, and 2.79 on D-100.

In this present study, there was a significant treatment by day interaction for Hct and Hgb

($P < 0.03$). From D-39 to D-72, GB, SB, and CON increased (GB: $P = 0.20$; SB: $P = 0.16$; CON:

$P = 0.02$) while SUG decreased ($P = 0.01$). All treatments had a decrease from D-72 to D-100,

although of differing degrees (GB: $P = 0.08$; SUG: $P = 0.41$; SB: $P = 0.01$; CON: $P = 0.008$).

This resulted in only SUG sows having a difference between D-39 and D-100 ($P = 0.008$).

Because SUG had a decrease from D-39 to D-72 while the other treatments had an increase,

SUG had lower Hct and Hgb values on D-72 than GB ($P = 0.06$), SB ($P = 0.02$), and CON ($P =$

0.004).

Sows on the GB treatment tended to have higher AnGap values than sows on the CON

treatment on D-100 ($P = 0.09$). Sows on the SUG treatment had lower AnGap values than sows

on the CON treatment on D-72 ($P = 0.049$). From D-39 to D-72, AnGap values decreased for

sows on the SUG treatment ($P = 0.03$).

Lipid Panel

Serum TOTch concentrations by treatment and day are shown in Figure 3.1. There were

treatment differences seen at mid- and late gestation (D-72 and D-100) with GB sows having

significantly higher TOTch than SUG sows (71.8 vs. 59.3 mg/dL and 64.3 vs. 52.9 mg/dL,

respectively; $P < 0.01$) and CON sows (71.8 vs. 64.1 and 64.3 vs. 53.6, respectively; $P < 0.03$).

Additionally, TOTch levels were also greater for GB than SB during mid gestation (71.8 vs.

61.5; $P = 0.006$), resulting in slightly higher TOTch for GB during late gestation (64.3 vs. 57.2;

$P = 0.08$). Higher overall TOTch for GB could be due to the greater concentrations of HDLch

present in GB sows compared to the other treatments.

Figure 3.1. Total serum cholesterol (mg/dL) over time for sows supplemented beef (GB), sugar
(SUG), combination of beef and sugar (SB), and a corn-soy control (CON) throughout gestation
and lactation.

Serum LDLch concentrations by treatment and day are shown in Figure 3.2. While there

were no treatment differences seen for circulating LDLch ($P > 0.05$), SUG sows did tend to have

greater LDLch in early gestation than CON and SB sows ($P = 0.05$ and 0.08, respectively).

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Serum LDLch decreased from early to late gestation for SUG sows ($P = 0.003$). For GB sows,

serum LDLch concentrations remained steady across gestation ($P > 0.64$). For CON sows, there

was an LDLch peak at mid-gestation compared to early and late gestation ($P = 0.04$), with no

difference between early and late gestation ($P = 0.89$). For SB sows, LDLch was the same for

early and mid-gestation ($P = 0.86$), but then tended to decrease in late gestation ($P = 0.09$ and

0.07 when compared to early and mid-gestation, respectively).

Figure 3.2. Total serum low-density lipoprotein cholesterol (mg/dL) over time for sows
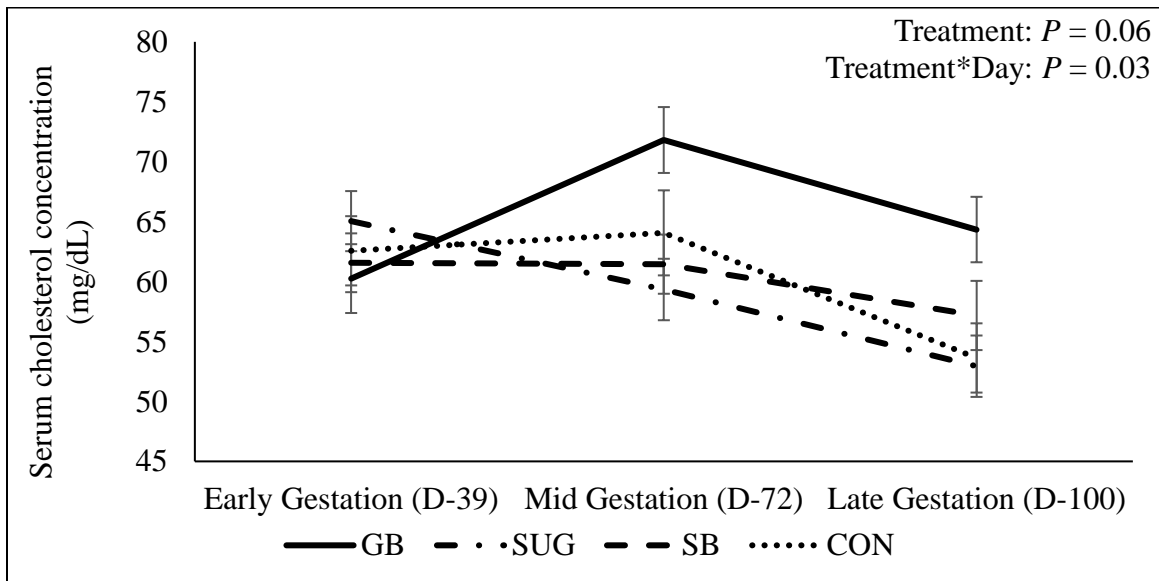supplemented beef (GB), sugar (SUG), combination of beef and sugar (SB), and a corn-soy
control (CON) throughout gestation and lactation.

Serum HDLch concentrations by treatment and day are shown in Figure 3.3. There were

treatment differences seen for HDLch with GB sows having the highest circulating HDLch

levels (24.4 mg/dL) when compared to SUG, CON, or SB sows (21.0, 21.9, and 22.2 mg/dL,

respectively) although only significant for SUG ($P = 0.004$) and only a tendency for CON or SB

($P = 0.052$ and 0.07, respectively). This difference is driven by D-72, or mid-gestation, levels

where GB sows had significantly higher HDLch concentrations than SUG, SB, or CON sows (P

< 0.02). There were no differences between treatments on D-39, or early gestation ($P > 0.64$). In

late gestation, GB sows had higher HDLch concentrations than SUG sows ($P = 0.04$), while

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there were no differences between other treatments ($P > 0.10$). Although HDLch concentrations

for GB sows increased from early to mid-gestation ($P = 0.30$) and then decreased from mid- to

late gestation ($P = 0.001$), all treatments saw an overall decrease in HDLch from early to late

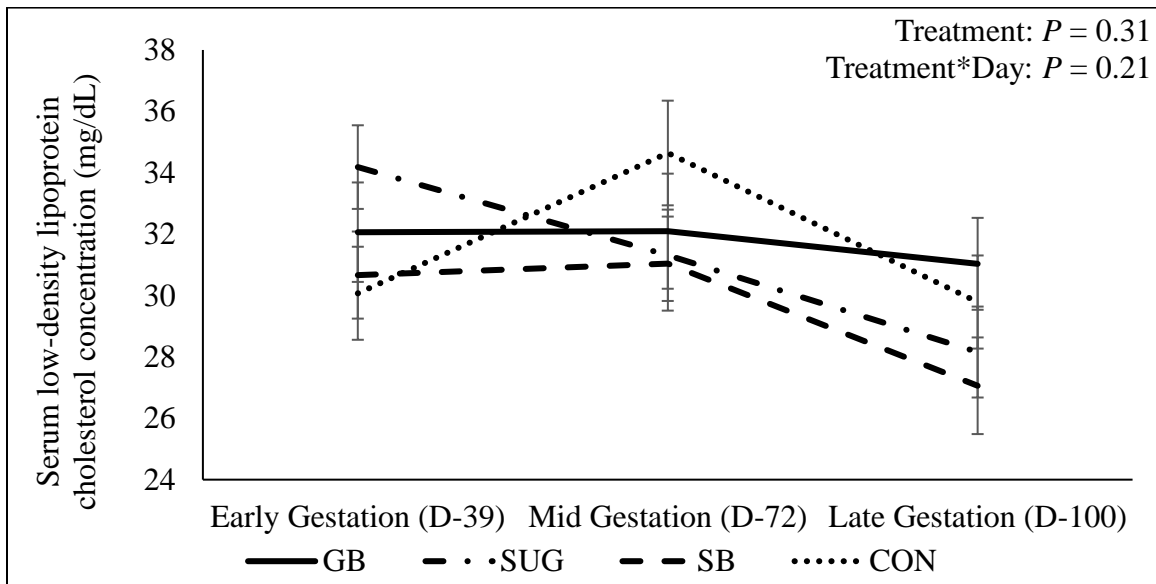gestation (GB: $P = 0.02$, SUG: $P < 0.0001$, SB: $P = 0.009$, CON: $P = 0.004$).

Figure 3.3. Total serum high-density lipoprotein cholesterol (mg/dL) over time for sows supplemented beef (GB), sugar (SUG), combination of beef and sugar (SB), and a corn-soy control (CON) throughout gestation and lactation.

Serum TG concentrations by treatment and day are shown in Figure 3.4. There were no

treatment differences seen in early ($P > 0.46$) or mid-gestation ($P > 0.38$). All treatments saw an

increase from early to late gestation (GB: $P = 0.02$, SUG: $P < 0.0001$, SB: $P = 0.009$, CON: $P <$

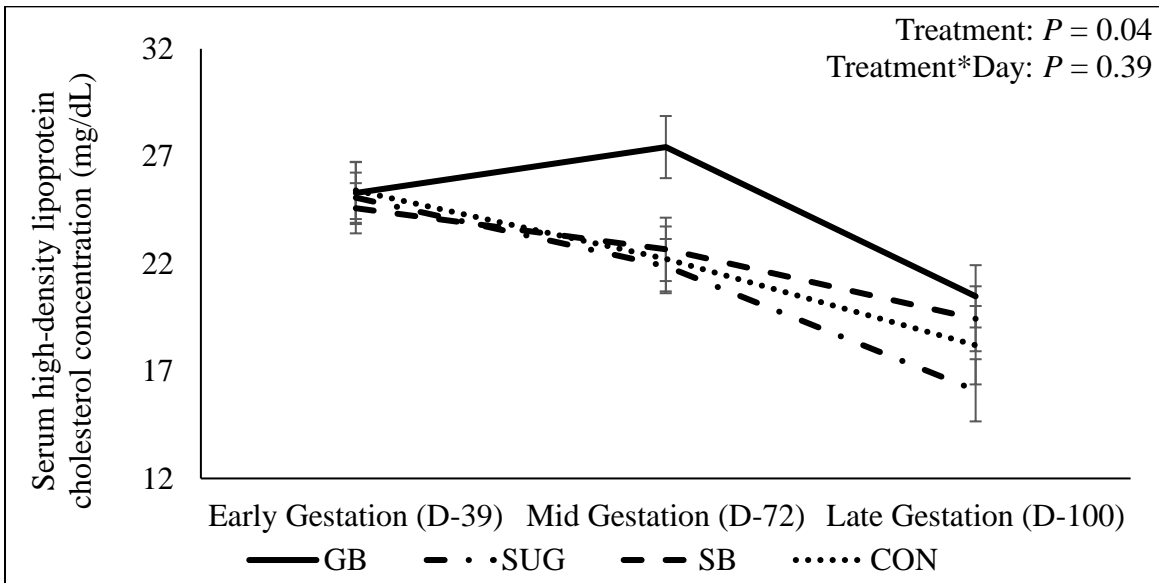0.0001). However, CON sows saw the greatest increase in serum TG concentrations, resulting in

a greater concentration in late gestation than GB, SUG, or SB sows ($P = 0.01$, 0.06, and 0.13,

respectively).

Figure 3.4. Total serum triglyceride concentrations (mg/dL) over time for sows supplemented beef (GB), sugar (SUG), combination of beef and sugar (SB), and a corn-soy control (CON) throughout gestation and lactation.

Serum Insulin

Serum insulin concentrations by treatment and day are shown in Figure 3.5. The only significant difference between treatments occurred in late gestation where CON sows had a lower serum insulin concentration than SUG sows ($P = 0.04$). There was a tendency for CON sows to have a lower concentration in mid-gestation than SUG sows ($P = 0.053$). There were no other differences between treatments in early, mid-, or late gestation ($P > 0.49$, 0.27, and 0.10, respectively). Serum insulin concentrations remained constant through gestation for GB sows ($P > 0.61$). Although not significant, SUG sows saw an increase in serum insulin levels from early to late gestation ($P = 0.19$). Both SB and CON sows had a numeric decrease in serum insulin levels from early to mid-gestation ($P = 0.23$ and 0.051, respectively) and a numeric increase in serum insulin levels from mid- to late gestation ($P = 0.34$ and 0.21, respectively).
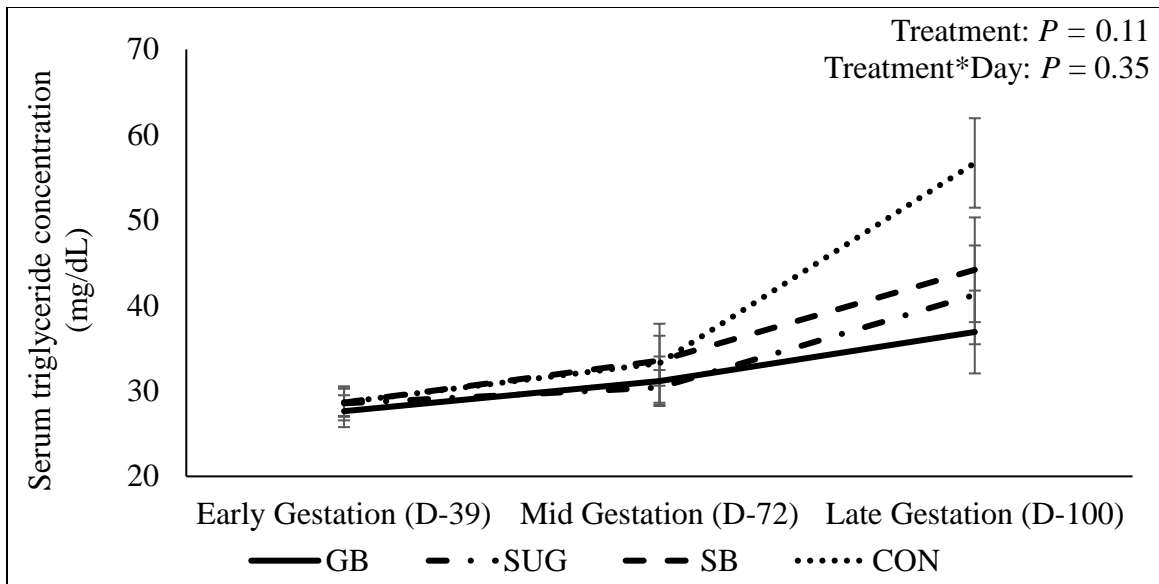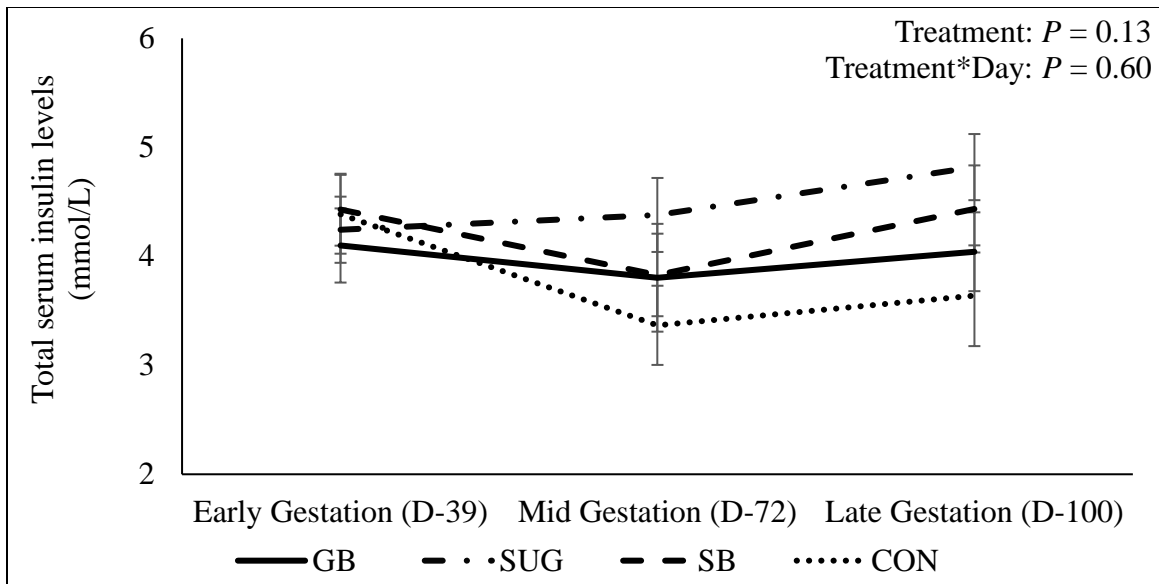
Figure 3.5. Total serum insulin (mmol/L) over time for sows supplemented beef (GB), sugar (SUG), combination of beef and sugar (SB), and a corn-soy control (CON) throughout gestation and lactation.

Milk Composition

Milk concentrations are shown by treatment in Figures 3.6 to 3.11. In this study, there were no treatment differences seen for percentage of fat, protein, lactose, MUN, and total and other solids ($P > 0.05$). When examining milk composition over time for all treatments, percent fat and percent protein decreased after D-0 (colostrum) and continued to remain relatively stable for the remainder of lactation (Figure 3.6 and 3.7). For all treatments, it was also observed that the percentage of lactose increased after D-0 and continued to remain stable throughout the remainder of lactation (Figure 3.8). When examining MUN, the percentage seemed to gradually decrease and increase during lactation with the lowest percentage being on D-18 (40.44 %; Figure 3.9). Lastly, the percentage of total solids (Figure 3.10) decreased while the percentage of other solids (Figure 3.11) increased after D-0 and remained constant.

Figure 3.6. Percentage of fat content from milk collected from sows supplemented beef (GB), sugar (SUG), combination of beef and sugar (SB), and a corn-soy control (CON) throughout gestation and lactation.
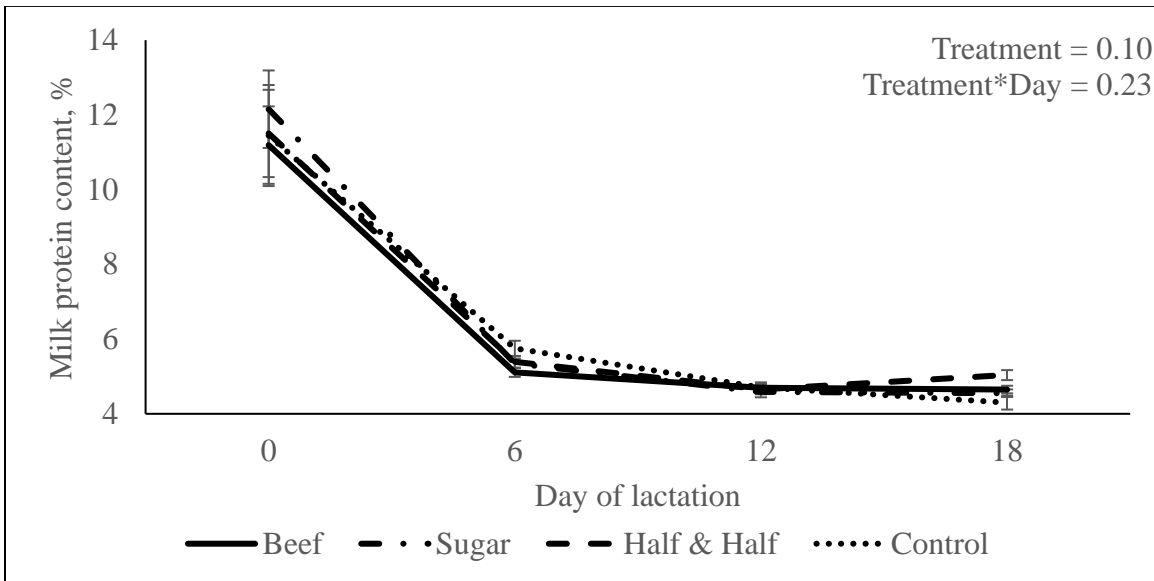
Figure 3.7. Percentage of protein content from milk collected from sows supplemented beef (GB), sugar (SUG), combination of beef and sugar (SB), and a corn-soy control (CON) throughout gestation and lactation.
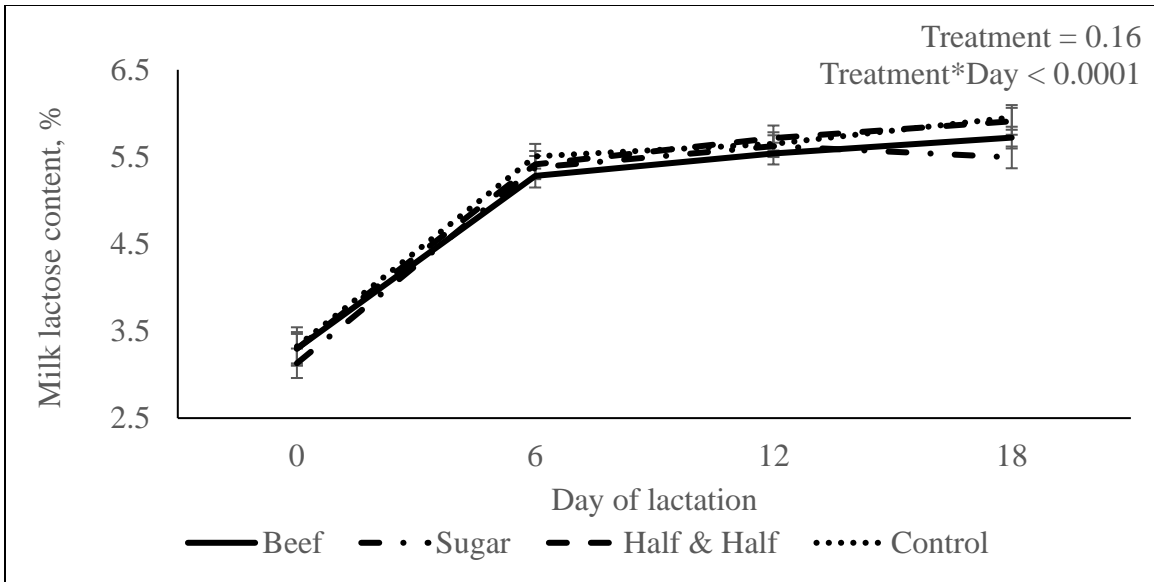
Figure 3.8. Percentage of lactose content from milk collected from sows supplemented beef (GB), sugar (SUG), combination of beef and sugar (SB), and a corn-soy control (CON) throughout gestation and lactation.
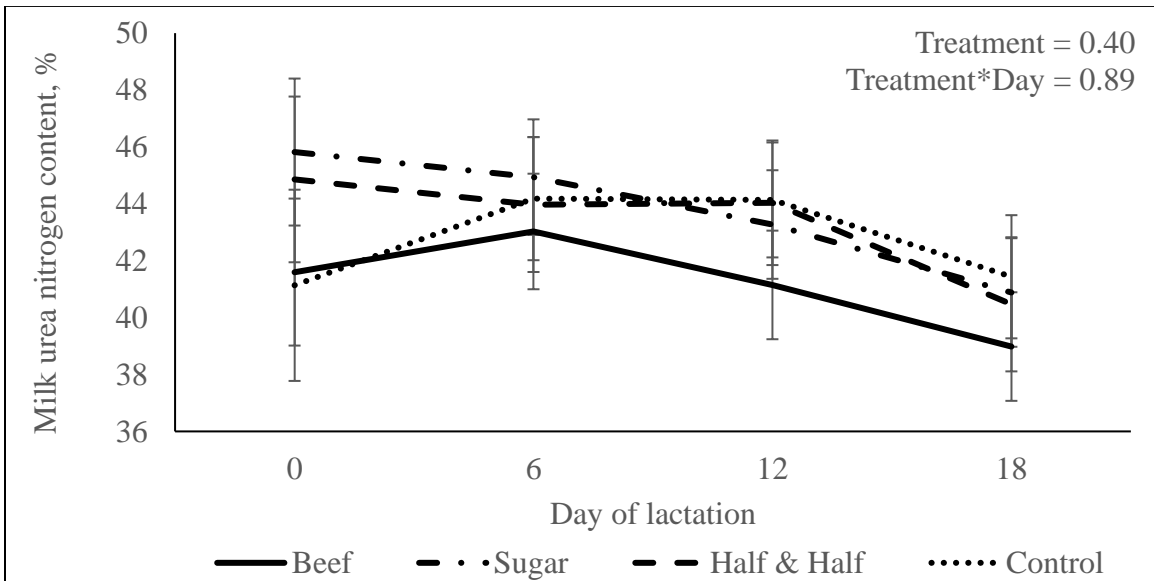
Figure 3.9. Percentage of milk urea nitrogen content from milk collected from sows supplemented beef (GB), sugar (SUG), combination of beef and sugar (SB), and a corn-soy control (CON) throughout gestation and lactation.

Figure 3.10. Percentage of total solids from milk collected from sows supplemented beef (GB), sugar (SUG), combination of beef and sugar (SB), and a corn-soy control (CON) throughout gestation and lactation.
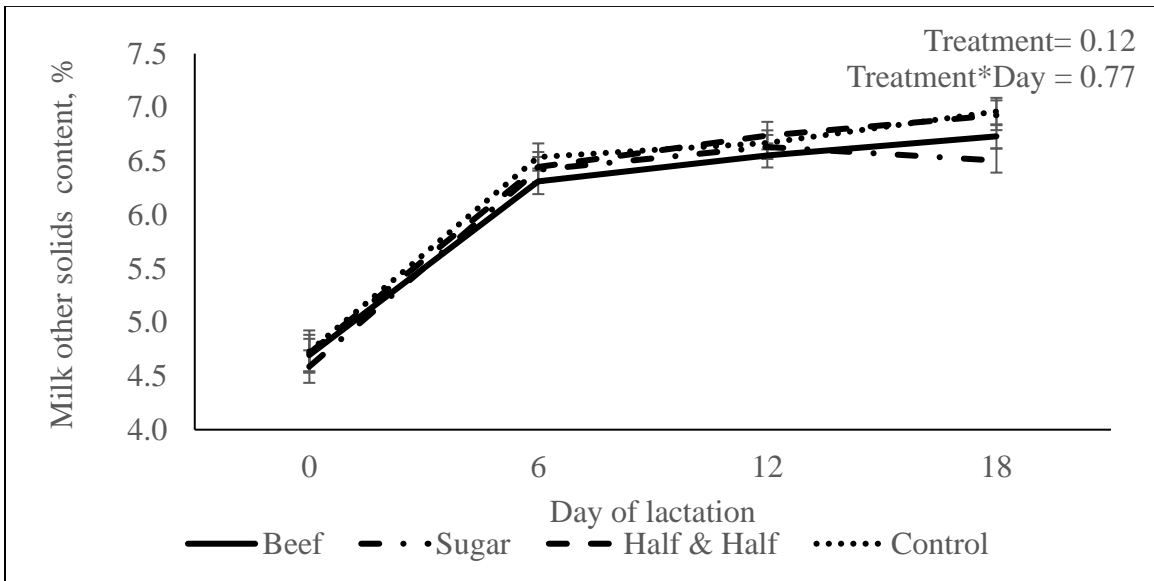
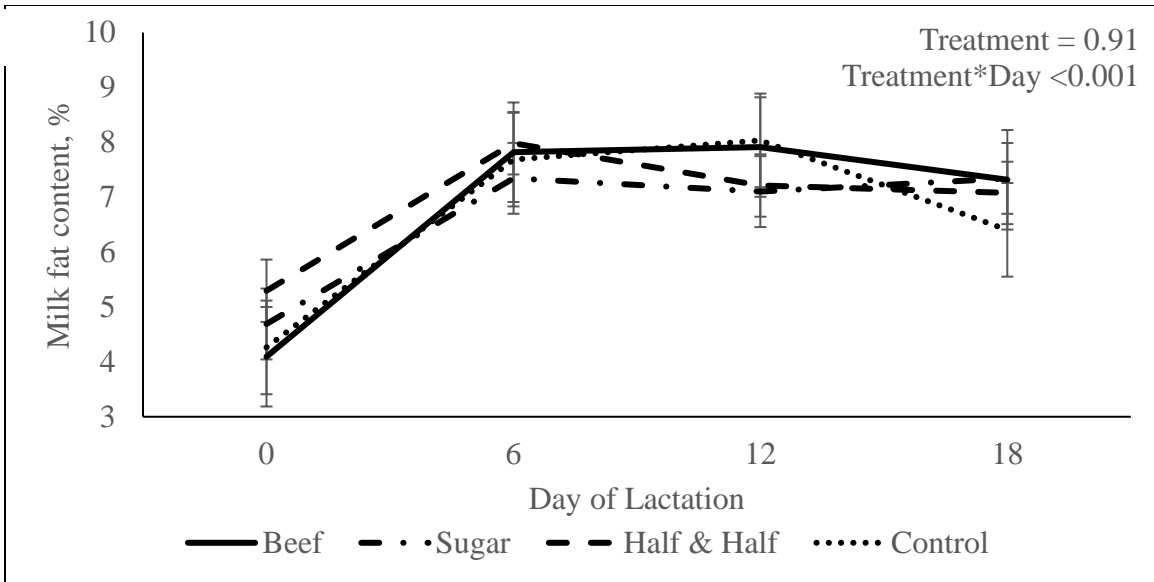**Figure 3.11.** Percentage of other solids from milk collected from sows supplemented beef (GB), sugar (SUG), combination of beef and sugar (SB), and a corn-soy control (CON) throughout gestation and lactation.
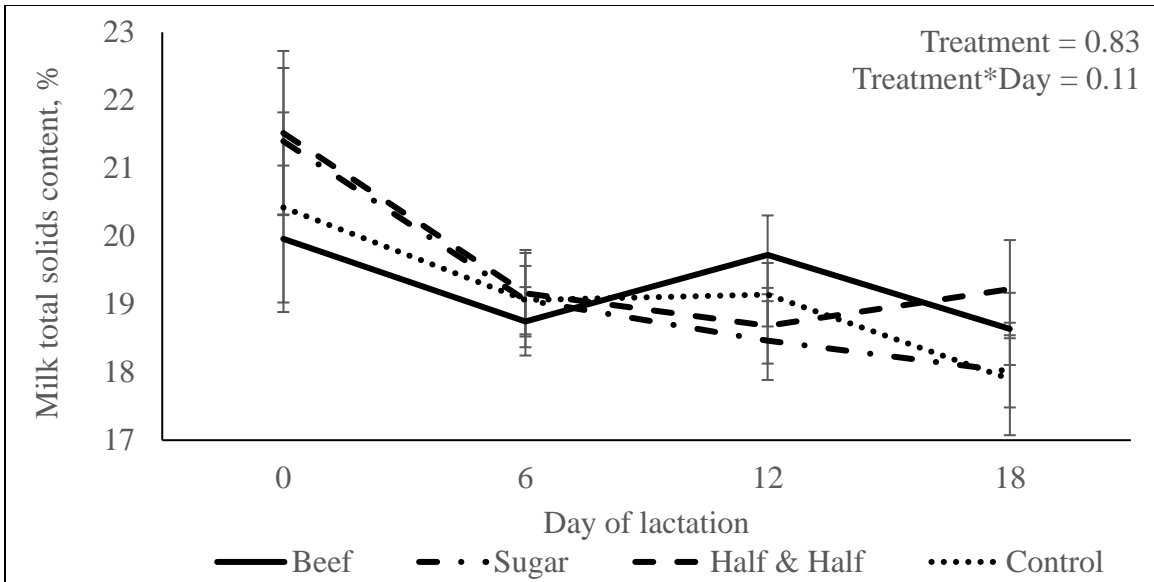
Discussion

Piglet Traits

In a study performed by Clouard et al. (2016) examining perinatal exposure to a diet high in fat, refined sugar, and cholesterol (**HFS**), litter size was not affected by the prenatal diets

when comparing HFS to control piglets. Prenatal diets also seemed to not effect body weight at birth; however, at weaning, HFS piglets weighed more than the control piglets (Clouard et al., 2016). Our present study saw similar to results to Clouard et al. (2016), as there was no effect on number of piglets farrowed when comparing GB, SB, or SUG perinatal dietary supplementation. In contrast to Clouard et al. (2016), this present study showed that GB dietary treatments had lower body weights at birth, D-6, and D-12. In our study, piglets exposed to the maternal dietary supplement of SUG were heavier at weaning than the piglets who were exposed to the CON supplement. While this was consistent with Law et al (2019) in regard to LGA babies whose mothers had higher blood sugar, the treatment impacted them early, but wasn't a big enough insult to keep going later in life.

Sow Traits

Interestingly, while there were no differences seen across treatments for sow traits, it was observed that the lactation efficiency of sows on the GB supplementation performed better when compared to the SUG supplemented sows. Similar to our findings, previous studies in lactating rats (Jansen and Monte, 1977; Naismith et al., 1982; Sampson and Jansen, 1985) and in swine (Mahan, 1977) indicated that protein intake can increase milk volume independently of total energy intake. Additionally, Rolls et al. (1980) conducted a study to investigate the effects of dietary supplementation on milk production and fed rats a high-energy, low-protein supplement in addition to their usual diet during lactation. It was found that although maternal food intake increased among the supplemented compared with the control animals, litter growth rate was reduced, indicating a reduction in milk output. Lastly, early studies in humans by Gopalan (1958) and Edozien et al. (1976) suggest the same relationship: milk output of women increased

when protein intake was increased from 50 to 60 g/day to approximately 100 g/day; however, increasing protein above 100 g/day caused no further change in milk volume.

Meat intake during pregnancy can provide important nutrients for both mother and fetus. While the relationship between intake of meat and weight gain have mixed beliefs, most studies have shown no weight changes observed when energy from carbohydrates were partially replaced with meat (Yamashita et al., 1998; Leslie et al., 2002; Hodgson et al., 2006; 23-26). When considering this current study, we saw similar results that weight changes were not observed due to added meat in the diet. Additionally, it has been suggested that a high protein diet may have potential beneficial effects because of increased satiety and thermogenesis (Paddon-Jones et al., 2008). While feed intake did not significantly differ across treatments, there was a numeric difference with GB and CON treatments consuming less total feed when compared to SB and SUG; potentially suggesting there may have been a satiety effect at play.

Blood Chemistry

iSTAT

Measuring BUN is important because it allows assessment of liver and kidney function. Urea is formed in the liver when proteins are metabolized and broken down into amino acids. It is carried by the blood within the body, filtered out by the kidneys, and removed from the body in the urine. If an individual's liver isn't healthy, it may not break down proteins the way it should. Additionally, if the kidneys aren't healthy, they may not properly filter out urea. There are many causes of elevated BUN and Creatinine levels with the most common causes including dehydration, urinary tract infection, consumption of high protein diets, and diabetic nephropathy (Kusina et al., 1999; Mokabberi and Ravakhah, 2007; Thomas et al., 2008; Beier et al., 2011). Elevated BUN level has been correlated with increased mortality in patients with acute heart

failure,(Hartz et al., 1995; Felker et al., 2004; Cauthe et al., 2008) chronic heart failure,(Filippatos et al., 2007) coronary artery bypass graft (Hartz et al., 1995) and is predictive for intensive care unit (ICU) stay and survival in acute necrotizing pancreatitis.(Faisst et al., 2009). Finally, BUN also predicts short term mortality following bone marrow transplant (Bacigalupo et al., 1999) and esophagectomy (Bailey et al., 2003).

A normal human BUN concentration spans 7 to 20 mg/dL. This is similar to BUN levels in swine which range from 7 to 14 mg/dL (UCDavis Veterinary Medicine, 2010; Mayo Clinic, 2018). A better measure is the ratio of BUN to creatinine found in blood. Typically, the ratio of BUN to creatinine should be between 10:1 and 20:1. If it's lower or higher than that, it may mean there are kidney problems present.

In this present study the BUN levels expressed for all dietary treatments were below the threshold for concern of relative risk for kidney damage. It is important to note that because urea is an end product of protein metabolism, diets that include a high-quality protein source will result in higher levels on BUN to be expressed (Beier et al., 2011). As a result, we expected to see an increase in the BUN concentration for the sow receiving the GB or SB dietary supplementations due to the higher protein content they were receiving in their diets. This present study also showed that creatinine levels remained constant across all dietary treatments which contradicted our belief that the creatinine levels would be significantly higher in the GB and SB dietary supplementations.

Hematocrit (Hct) and hemoglobin (Hb) readings are used as a means to diagnose anemia. The Mayo Clinic defines Hct as the proportion, by volume, of the blood that consists of red blood cells, expressed as a percentage (Mayo Clinic, 2016a) while Hb is the protein molecule in red blood cells that carries oxygen from the lungs to the tissues and returns carbon dioxide from

these tissues back to the lungs to be exhaled. According to The Veterinary Technician's Large Animal Daily Reference Guide, normal swine levels for Hb range from 10 to 16 g/dL while the Hct normal range is 32 to 50 % (D'Andrea and Sjogren, 2013).

In this present study, it was expected that the GB supplementations provided to the sows would have greater circulating Hct and Hb concentrations due to the higher concentrations of heme-iron and vitamin B-12 in red meat; which ultimately play a role in Hct and Hb levels. Concentrations of Hb ad Hct typically decrease during the first trimester and reach the lowest levels at the end of second trimester and increase again during the third trimester of pregnancy (Laflame and McGill, 2011; Moghaddam Tabrizi and Barjasteh, 2015). While Hct and Hb concentrations remained stable throughout this present study, it was clear that the GB sows have higher concentrations of both Hb and Hct throughout the duration of the study.

Glucose is a major energy substrate for fetuses and has been shown to decrease in sows with large litters, due to the high-energy requirement necessary to sustain the uterus and fetuses during gestation, and blood glucose can provide precursors to mammary glands for milk synthesis during lactation, respectively (Père et al., 2000; Park et al., 2010). Therefore, due to the added glucose content in the SUG dietary supplementation in our present study, this may reduce the negative energy balance and may improve sow body condition during lactation. Moreover, there is inadequate evidence available suggesting additional glucose has a positive or negative influence on the sow's fertility, the number of liveborn piglets or birth weight of the piglets. And no studies have been conducted to evaluate the effects of dietary glucose inclusion on sow performance and milk composition. Therefore, it is important to continue to investigate the effects of different dietary glucose inclusion during lactation on body condition, reproductive performance, milk composition, blood chemistry in sows.

Cholesterol is a compound of the sterol type found in most body tissues. It is an essential structural component of cell membranes, affecting their stability and permeability. Additionally, cholesterol is essential as the precursor of the sex and adrenal steroid hormones and of the bile acids that play important roles in lipid digestion and absorption (Stipanuk and Caudill 2013). Both synthesis and disposal of cholesterol must be tightly regulated to ensure needs are met and to prevent excess accumulation.

High cholesterol levels during pregnancy are necessary to make steroid hormones, such as progesterone and estrogen, which are vital for maintaining pregnancy and initiating parturition respectively. Cholesterol levels increase naturally during the second trimester, peak during the third and typically return to normal about four weeks after delivery (Di Cianni et al, 2003; Lepsch et al., 2017). During pregnancy, LDLch levels should be less than 100 mg/dL and the HDLch should be greater than 60 mg/dL. Furthermore, during pregnancy, overall cholesterol levels can increase by 25 to 50 percent, and fortunately, HDL levels seem to increase more (Lain and Catalano, 2007; Farias et al., 2016). When examining the results from this present study, while there were treatment differences seen, the overall trends of when cholesterol leaves peaked and decreased followed what would be considered normal during pregnancy. However, HDLch levels for swine in this study were generally lower than the recommend normal values of 60 mg/dL or higher during pregnancy regardless of treatment.

Previous research done by Welsh et al. (2010) found correlations between dietary added sugars and blood lipid levels showing that lower HDLch and higher TGs were associated with higher consumption of added sugars. In our current study, similar results were seen with the sows consuming the SUG dietary supplement displaying decreasing circulating HDLch

throughout gestation and having the lowest HDLch during late gestation when compared to the other three dietary treatments. Additionally, similar results were seen with circulating TGs levels, with SUG sows TGs levels increasing throughout gestation and possessing the highest circulating TGs during late gestation when compared to GB, SB and CON treatments.

The added sugar intake and changes in LDL, HDL, and triglycerides may lead to the increased risk of coronary heart disease (Te Morenga et al., 2014; DiNicolantonio et al., 2015.). However, it is important to note that during pregnancy there is an increased production of sex steroids and the increased amount of progesterone concentration can contribute to the rise in LDL levels (Chiang et al., 1995). Therefore, the altered lipid metabolism of LDLch and HDLch that was seen in the present study also could have been contributed by the accumulation of maternal fat stores in the first half and enhanced fat mobilization in the second half of gestation (Butte, 2000).

Milk Composition

There are many components to milk including proteins, lipids, carbohydrates, minerals, vitamins, and cells. Additionally, milk composition can be affected by a variety of factors, with stage of lactation having the most dramatic influence on composition (Hurley, 2015). Mammary secretions from sows during the initial 24 h post-farrowing are generally higher in concentrations of immunoglobulins, some microminerals and vitamins, hormones, and growth factors, but are lower in concentrations of lactose, when compared with mature milk (Salcedo et al., 2016; Theil and Hurley, 2016). Fat concentration in sow milk is transiently increased from day two to day four of lactation. The composition of milk after day 7 to day 10 is relatively stable for the remainder of lactation. The composition of milk also varies with the fraction that is removed during milk ejection, with the hind milk having higher concentrations of total solids, fat, and

energy, but not protein and lactose, compared with the fore milk fraction (Atwood and Hartmann, 1992). Some studies have found no effect of feeding supplemental animal fat on milk total solids and other studies have found that supplementation of dietary fat increased milk fat percentage in sow mammary secretion (Klobasa et al., 1987; Averette et al., 1999; King'ori, 2012). However, Kusina et al. (1999) found that neither dietary crude protein intake nor dietary lysine level affected sow milk fat.

Contrary to our beliefs, our present study showed no difference in milk composition across dietary treatments. Neither the addition of ground beef or sugar had a positive or negative impact on the milk composition; therefore, not altering the nutrient content of the milk the piglets were suckling across sow dietary treatments. It was also interesting that milk urea nitrogen concentration did not differ from the GB supplementation when compared to the other three treatments. Milk urea nitrogen reflects the amount of urea found in milk and these values are closely correlated with the concentration of urea found in the blood, for which we saw BUN values were greatest for sows on the GB supplementation.

Additionally, it has been previously reported that milk composition can be affected by oxytocin administration, where intravenous administrations resulted in lower concentrations of total solids, fat, lactose and energy when compared with manual stimulation or intramuscular oxytocin administration (Hurley, 2015). However, the route of administration will not affect protein content within the milk composition. All of our oxytocin was administered intramuscular; however, further research should be done to compare route of administration on milk composition. Furthermore, more research is needed to examine how much sugar or beef is needed to be consumed to alter milk composition of nursing sows.

Conclusion and Implications

In both animal and human studies, it is known that altering maternal and fetal metabolic environment can affect the overall health of both the mother and child. Obesity has become an epidemic and more women at a reproductive age are being classified as obese. Moreover, obesity during pregnancy is also associated with GDM as well as other health complication, which can increase the risk factor for development of childhood obesity, diabetes and metabolic syndrome. Superior food quality, together with adequate macro- and micronutrient intake during pregnancy, is crucial for the health status of the mother and child.

In this present study, while there were differences seen in TOTch and HDLch for the sows, these variations in lipids followed what was to be expected during pregnancy. Additionally, the higher levels of the blood components BUN, Crea, Hct and Hb present in the GB treatment can be explained by the increased protein consumption from the added beef within the diets. While there were differences seen for number of piglets born, dead at birth, as well as fully formed piglets, upon reaching D-6 of life there were no significant differences seen in growth of the offspring across treatment. Milk composition did not differ across treatment for dietary supplementation; therefore, all piglets across treatments were receiving milk of similar chemical composition and did not play a role in altering early growth and development of offspring.

When taking into consideration the size of the sows and the small percentage of the diet that came from the supplementations, a greater amount of added protein as beef or added sugar may have been necessary to observe more health altering effects. Additionally, this study showed that when ingesting beef or sugar on a caloric basis, adverse offspring development and growth was not present; ultimately showing that there was no difference between the supplementations

and health. By increasing the amounts of added meat and sugar in the diet there is potential for further research to be conducted on the effects of red meat being a main source of protein during pregnancy and how it may be beneficial to maternal health and early offspring development. Future research should also be done to investigate the effects of gestational weight gain on offspring development. When considering lipid panels, and high cholesterol being a concern in public health, research to be considered would be on how to better evaluate TOTch, HDLch, LDLch and TGs, during pregnancy to identify a means to better monitor lipid panel changes to ensure these changes remain within normal ranges for pregnancy.

Furthermore, it can be concluded that the total diet intake, including the usual diet and the supplement, provided during gestation and lactation had neither a positive nor negative effect on overall maternal health and early offspring development and growth. Positive outcomes in the offspring and mothers cannot be expected if the supplementation is not needed. There is no consensus on the best time to change dietary patterns during pregnancy. While most of the fetal weight gain occurs in the third trimester, nutritional status of woman during the preconception period may be a more important determinant of fetal growth than nutritional status during the latter part of pregnancy. Future research examining individuals who benefit from supplementation, different time periods to start supplementations, as well as varying types and amounts of supplements are warranted.

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CHAPTER 4. THE EFFECTS OF FEEDING BEEF AND SUGAR SUPPLEMENTATIONS THROUGHOUT PREGNACY AND LACATION IN A SWINE BIOMEDICAL MODEL ON OFFSPRING MATURE BODY SIZE AND COMPOSITION[2]

Abstract

Using swine as a model for humans, the objectives of this project were to determine if supplementations of ground beef (**GB**), sugar (**SUG**), or a combination of the two (**SB**) to sows during gestation and lactation would affect offspring mature body size and composition. At weaning, the average male and female from each litter across treatments was determined and transported to the NDSU Animal Nutrition and Physiology Center (Fargo, ND). There were differences seen between sexes with boars having heavier pre-rigor carcass weights (**PCW**) when compared to gilts ($P < 0.0001$). Additionally, there were treatment by sex differences seen with GB boars and CON boars having heavier PCW when compared with SB males (P =0.03; P=0.01). Higher dressing percentages were observed for boars when compared to gilts (P =0.0004). There was no treatment effect observed on the proportional size of the liver, pancreas, heart, lungs, or longissimus dorsi muscle area ($P > 0.05$). While not significant, there was a tendency seen for SUG offspring to have heavier testicular weights when compared to the other three treatments ($P = 0.08$). Lastly, there was a sex difference seen for subjective color and

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[2] The material in this chapter was co-authored by A. M. Siomka, A. Hoyle, J. M. Young, A. Ward, and E. Berg. A. M. Siomka was responsible for animal husbandry, piglet processing, weighing and weaning. A. Hoyle was responsible for animal husbandry and piglet care post-weaning, weighing pigs, performing carcass ultrasounds, blood collection, and assisted in tissue collect at harvest. J. M. Young was responsible for animal husbandry and piglet care pre-weaning, statistical analysis and proofreading. A. Ward was responsible for study design, assisted with slaughter and tissue collections E. Berg was responsible study designed, assisted with slaughter and tissues collections, assisted with statistical analyses, and proofreading.

marbling scores, with gilts possessing dark longissimus dorsi muscle and more marbling when compared to boars.

Introduction

Maternal nutrition during pregnancy and lactation has been shown to affect mature body composition (Armitage et al., 2004; Wu et al., 2004; Maslova et al., 2014). The amount and quality of food that the mother consumes during gestation influences birth weight and susceptibility of progeny to disease in later life (Parlee and MacDouglad, 2013). In recent years, epidemiological, clinical, and basic research has reported interrelationships between fetal nutrition, adipose tissue development, central control of energy balance, and the propensity for obesity in adult life (Parlee and MacDouglad, 2013). Rehfeoldt et al. (2012) found that offspring exposed to high protein during gestation and lactation showed a decrease in subtypes of body fat. Results from animal studies performed by Thone-Reinke et al. (2006) and Vester et al. (2009) found that high protein consumption, in the form of meat, during gestation and lactation had limited effect on glucose and lipid metabolism.

Additionally, in relation to GDM, it has been shown that increased intrauterine exposure to glucose may stimulate greater insulin secretions form the fetus, influence epigenetic modifications, alter the developmental programming of appetite control, modulate the child's energy balance system, and affect adipocyte metabolism (Lawlor, 2013; Sharp et al., 2015). Furthermore, these early life changes in the offspring may lead to the development of obesity and adverse cardiometabolic health later in life (Engl, 2013; Lawlor, 2013; Li et al., 2015).

The objective of this study was to examine the effects of maternal nutrition in the form of added sugar and protein (red meat) during pregnancy and lactation on offspring mature body weights and composition. The hypothesis of this study is that the intake of added sugar during

pregnancy and throughout lactation will cause offspring to possess heavier body weights, more adipose tissue, and an increased risk of offspring developing obesity through their life course.

Materials and Methods

This study was conducted at the North Dakota State University (**NDSU**) Swine Research Unit, the NDSU Animal Nutrition and Physiology Center (**ANPC**), and the NDSU Meats Laboratory (Fargo, ND). All animal care and handling procedures were approved by the Institutional Animal Care and Use Committee (protocol # A17010) under the supervision of the attending veterinarian.

Animals and Housing

Purebred Yorkshire and crossbred Chester White x Yorkshire sows of at least 14 mo of age to ensure that the majority of developmental growth had occurred were selected to minimize genetic variation amongst the sows and to utilize maternal breeds for improved reproduction traits. Thirty-five sows over 4 repetitions were utilized. Two full-sibling Hampshire x Duroc boars were used as sires to minimize genetic variation in the offspring. At approximately D-30 post-insemination, sows were confirmed pregnant using a portable ultrasound machine (Veterinary Ultrasound Scanner, Model 8300 Class I Type B) and moved into the gestational research room and placed into individual gestation stalls (84" L x 22" W x 48.75" H) with nipple type waterers. Day-length was standardized across repetitions by blocking all-natural light from the room, turning lights on at 0700, and turning lights off at 1900. Sows were provided with music (when the lights were on) and chains hung at nose level to stimulate artificial rooting behavior as a means of environmental enrichment. They also had nose-to-nose contact with the neighboring sows.

Sows were fed a standard gestation diet (Table 4.1) formulated to meet the nutritional guidelines provided by the National Research Council (NRC, 1998). Sows were fed 2.04 kg of the standard diet at 0700 each day. Adjustments were made to this according to changes in body condition. An overly conditioned sow (body condition score of 5) was fed 1.81 kg each day. An under conditioned sow (body condition score of 1) was fed 2.27 kg each day. D- 40 of gestation, sows were randomly assigned to 1 of 4 supplementation treatments: 1) Beef (**GB**): a 4 oz cooked beef patty (Food Service Direct, Hampton, VA); 2) Sugar (**SUG**): table sugar at a caloric equivalent to the 4 oz beef patty; 3) Half sugar and half beef (**SB**): half of a 4 oz cooked beef patty and table sugar at a caloric equivalent to 2 oz of the beef patty; and 4) Control (**CON**): standard gestation diet at a caloric equivalent to the 4 oz beef patty. Supplements were given at 1100, 1500, and 1900 each day. Sows were provided *ad libitum* access to water.

Table 4.1. Ingredient composition and formulated nutrition content of the basal diet (as-fed basis).

Ingredients[1]	Gestation	Lactation
Corn	70.80	71.60
Soybean Meal	9.85	23.35
Soybean Hulls	15.00	0.00
Monocalcium	1.47	1.42
Limestone	1.06	1.10
Fat	0.75	1.25
EnMax Sow Premix 10[2]	0.50	0.50
Salt	0.45	0.45
Choline 60 (Dry)	0.12	0.12
L-Lysine	0.00	0.12
L-Threonine	0.00	0.06
DL-Methionine	0.00	0.03
Nutrient		
Net Energy, Mcal/kg	2.31	2.42
Crude Protein, %	11.22	16.02
Lysine, %	0.70	1.10
Calcium, %	0.78	0.80
Phosphorus, %	0.60	0.65

[1] Ingredients are given on a percent basis of the total ration.
[2] Preformulated vitamin/mineral mix produced by Ralco Nutrition

At D-110 of gestation, sows were moved from the gestational research room to the farrowing room. At this time, sows were switched to a lactation diet (Table 4.1) and fed 1.36 kg feed/204 kg of body weight at 0700 and 1500 daily. Supplements were still fed at 1100, 1500, and 1900. Once sows farrowed, feed intake was increased incrementally to a maximum of 3.63 kg feed/204 kg of body weight at each feeding.

Once farrowing was completed, standard D-0 processing for the NDSU Swine Research Unit was conducted and farrowing date recorded. Sex, birth weight, and teat count were recorded. Piglets were ear notched for identification and needle teeth clipped. On D-2 post-farrowing, standard D-2 processing for the NDSU Swine Research Unit was conducted with one exception. Piglets had their tails docked and were given 1 cc each of iron (Uniferon 200, Pharmacosmos, Inc., Watchung, NJ) and Duramycin 72-200 (Durvet, Inc., Blue Springs, MO) intramuscularly on opposite sides of the neck. Because this project was biomedical in nature, male pigs were not castrated at this time. At weaning (D- 18 of age), piglets were given 1 cc of Bo-Se (Merck Animal Health Intervet Inc., Madison, NJ) and 2 cc of RhiniShield TX4 (Elanco U.S., Inc., Larchwood, IA) intramuscularly on opposite sides of the neck. One-week post-weaning, piglets were given 1 cc each of MycoFlex (Boehringer Ingelheim Animal Health USA Inc., Duluth, GA) and CircoFlex (Boehringer Ingelheim Animal Health USA Inc., Duluth, GA) intramuscularly on opposite sides of the neck. The median boar and gilt from each litter were determined at weaning and transported 2.6 km to NDSU ANPC nursery room.

Upon arrival, piglets were placed into individual pens (1.22 x 2.44 m) with gilts and boars separated on opposite sides of the room. Room temperature was maintained at 29.4 °C for ~2 wk, 26.7 °C for ~1.5 wk, and 23.9 °C for the remainder of the time in that room. Temperature was recorded daily at 0700 at both ends of the room. Each pig was provided with a bocce ball as

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a means of environmental enrichment in addition to nose-to-nose contact with their neighboring

pig.

At approximately 27 kg (or 60 d of age), all pigs were moved into a growing rooming,

where they were once again individually housed (36.5" L x 57.5" W x 43.0" H) and separated on

opposite sides of room based on sex (boars vs. gilts). Environmental enrichment was provided as

nose-to-nose contact and music. The growing room temperature was kept at approximately 21.1

°C and temperature was recorded daily. Once the pigs reached approximately 54.4 kg (or ~120 d

of age), all animals were moved to a third and final room, known as the finishing room. Pigs

Table 4.2. Ingredient composition and formulated nutrition content for swine nursery and grow-finish diets for swine (as-fed basis).

No DDGS	Nursery Diets			Grow-Finish Diets		
Ingredients	ND1	ND2	ND3	GFD1	GFD2	GFD3
Corn	576.0	1048.4	1409.65	1534.2	1627.6	1692.2
Soybean Meal	324.0	478.0	528.0	414.0	330.0	271.0
Monocal	0.0	10.9	14.4	9.5	4.4	1.9
Limestone	0.0	13.4	21.2	19.0	17.8	17.2
Fat, CWG	30	0.0	0.0	0.0	0.0	0.0
5512 EnMax Sow Premix 10	0.0	6.0	5.0	0.0	0.0	0.0
5490 EnMax Ralco Starter Nursery Base 1100	1100.0	400.0	0.0	0.0	0.0	0.0
5509 EnMax GF Premix 7.5	0.0	0.0	3.75	7.5	7.5	7.5
Salt	0.0	8.0	9.0	8.0	8.0	8.0
L-Lysine	0.0	3.0	5.2	5.0	4.0	2.2
L-Threonine	0.0	0.9	1.6	1.3	0.5	0.0
DL-Methionine	0.0	1.4	2.2	1.5	0.2	0.0
Nutrient						
Net Energy, Mcal/kg	2.35	2.40	2.37	2.45		
Crude Protein, %	22.45	19.65	17.53	15.31		
Lysine, %	1.60	1.42	1.30	1.15		
Calcium, %	0.70	0.70	0.67	0.56		
Phosphorus, %	0.64	0.58	0.52	0.45		

were individually housed (75" L x 90" W x 37" H) and separated by sex. Music was provided as environmental enrichment. The temperature was kept at approximately 18.3 °C and recorded daily.

Upon reaching ANPC, all piglets were fed a standard nursery diet using the National Research Council dietary recommendations for growing swine (NRC, 1998) and then phase-fed for ~5.5 mo. While in the nursery, pigs were fed 3 diet phases (Table 4.2). In the grower room, pigs were fed the first 2 grow-finish diet phases (Table 4.2). Pigs were transitioned to the finishing room while on the second grow-finish diet phase and then fed the third grow-finish diet phase (Table 4.2). Pigs were fed twice a day at 0700 and 1500. All pigs were provided *ad libitum* access to water.

Body Composition

Every two weeks, BW were obtained staring at d28 to monitor growth, average daily gain (**ADG**), ADFI, and G:F for each pig. Additionally, Aloka, an SSD-500V ultrasound machine fitted with a 3.5-MHz, 12.5-cm, linear array transducer (Corometrics Medical Systems, Inc., Wallingford, CT) operated by a trained ultrasound technician was used to obtain subcutaneous fat depth (**FD**) and longissimus muscle area (**LMA**) at the 10th thoracic vertebra on d84 and then every 14 d. These data were used to quantify growth curve analysis across treatments. Total fat-free lean body mass (**FFL%**) was calculated from an equation using FD, LMA, and live BW (Baas et al., 2000) and expressed as a percentage of BW. Weights were taking every 2 weeks after d28 blood collections; being the first weight after weaning. Ultra-sound measurements began on d84 when all pigs were at least 45.4 kg. Ultra-sound collections were obtained on the same days as weighing for each pig. Both continued till completion of the trial.

Postmortem Evaluation and Tissue Collections

Pigs were humanely euthanized and processed under USDA Food Safety and Inspection Service Guidelines. Pre-rigor carcass weights (**PCW**) were recorded, and a modified necropsy was performed. Weights were obtained for heart, perirenal fat, liver, pancreas, kidneys, and uterus. Ovary and testicular weight were taken. Subcutaneous fat depth and examination of intramuscular fat was obtained at the juncture of the 10th and 11th thoracic vertebra. Additionally, subjective colors scores of the longissimus dorsi were taken at the same anatomical location. Liver and kidney were also measured for L*, a*, or b* color scores.

Subjective Intra-muscular Fat and Color Score Measurements

Intra-muscular fat (IMF) and color of the longissimus dorsi muscles were visually assessed, subjectively. Before subjective IMF and color scores were taken, the surfaces of the freshly cut muscles were exposed to air for at least 15 minutes prior to scoring. A trained person using the color scale developed by the National Pork Board was used. Color scores on the scale ranged from 1 to 6, with 1 being the lightest and 6 being the darkest in color. Intra-muscular fat scores were also measured utilising The National Pork board standard scale. Intra-muscular fat was measured on a similar scale of 1 (practically no IMF) to 10 (abundant IMF), using the National Pork Board standard marbling (IMF) scale.

Statistical Analysis

Analyses were conducted using mixed procedure in SAS (v. 9.4, SAS Institute, Cary, NC). The model included fixed effects of repetition, sex, and treatment. Two- and three- way interactions between fixed effects were tested and removed from the model if $P > 0.1$. Because treatment was applied to the sow, sow nested within repetition was fit as the subject of a repeated measures statement. Since some sows were used for multiple reps, sow was also fit as a random

effect. Different covariance structures were tested for the repeated measures and best fit based on AICc was chosen.

Results

There was a sex by treatment interaction for PCW ($P = 0.08$; Figure 4.1), with no differences seen between treatments for gilts ($P > 0.24$). However, GB and CON boars had greater PCW than SB boars ($P = 0.03$ and 0.01, respectively). While SB boars did not differ from SB gilts ($P = 0.99$), GB, SUG, and CON boars all had greater PCW than their respective gilts ($P < 0.002$). When examining pre-rigor carcass weight expressed as a percentage of live weight (dressing percentage; **DP**), gilts had a higher DP compared to boars ($P = 0.0004$; Table 4.3). While treatment was not significant for DP ($P = 0.35$), GB offspring tended to have lower DP than CON offspring ($P = 0.09$). There were no significant sex or treatment differences observed for pH at the 10th rib or last lumbar vertebrae (Table 4.3). However, SB offspring tended to have lower pH at the last lumbar vertebrae than CON offspring ($P = 0.06$).
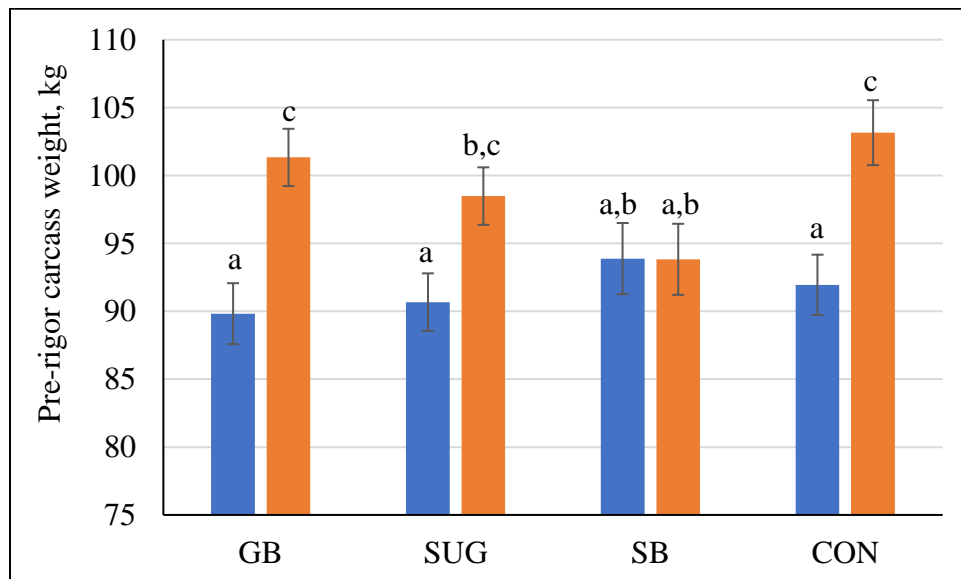

Figure 4.1. Least square means for pre-rigor carcass weight by treatment (supplementation of GB = ground beef, SUG = sugar, SB = combination of ground beef and sugar, and CON = corn-soy) for gilts (blue bars) and boars (orange bars). Bars with different letters (a,b,c) differ by $P < 0.05$.

Table 4.3. Least square means (standard error) by sex and treatment (**TRT**) of pre-rigor carcass weight (**PCW**), dressing percentage (**DP**), and pH at the last lumbar and 10th rib for offspring whose mothers were fed cooked ground beef (**GB**), sugar (**SUG**), a combination of cooked ground beef and sugar (**SB**), or a control of corn-soy (**CON**) supplementation through gestation and lactation.

Trait	Treatment				Sex[1]		P-value	
	GB	SUG	SB	CON	F	M	Sex	TRT
DP, %	77.43	77.99	78.34	78.51	78.72^a	77.42^b	<0.001	0.35
	(0.43)	(0.39)	(0.51)	(0.46)	(0.29)	(0.28)		
45 min pH at last	6.39	6.37	6.18	6.48	6.45	6.27	0.11	0.30
lumbar vertebra	(0.10)	(0.10)	(0.12)	(0.11)	(0.10)	(0.10)		
45 min pH at the	6.32	6.32	6.30	6.23	6.35	6.26	0.33	0.98
10th rib	(0.10)	(0.10)	(0.10)	(0.10)	(0.06)	(0.07)		

[1] F = female; M = male.
^{a,b} Values with a different superscript within a main effect (treatment or sex) differ by $P < 0.05$.

Table 4.4. Least square means (standard error) by sex and treatment (**TRT**) of organ weights for offspring whose mothers were fed cooked ground beef (**GB**), sugar (**SUG**), a combination of cooked ground beef and sugar (**SB**), or a control of corn-soy (**CON**) supplementation through gestation and lactation.

Trait	Treatment				Sex[1]		P-value	
	GB	SUG	SB	CON	F	M	Sex	TRT
Pancreas, g	160.0	163.3	161.0	160.4	166.5	155.9	0.18	0.99
	(7.3)	(6.6)	(8.5)	(7.9)	(5.5)	(5.4)		
Liver weight, g	1396.7	1495.6	1446.5	1463.7	1374.8^a	1526.5^{bl}	0.005	0.33
	(39.3)	(35.8)	(46.3)	(43.9)	(33.2)	(32.0)		
Heart weight,	433.4	464.2	460.6	444.5	426.7	464.7	0.28	0.52
with aorta, g	(15.6)	(14.0)	(18.2)	(18.3)	(15.4)	(14.7)		
Heart fat	74.6	78.9	79.5	62.1	103.3^a	44.3^b	0.002	0.65
weight, g	(10.1)	(8.2)	(12.1)	(11.1)	(10.7)	(9.7)		
Lung weight, g	889.4^{ab}	1036.4^b	817.3^a	820.1^a	926.3	860.2	0.34	0.01
	(52.5)	(47.8)	(61.8)	(58.6)	(44.3)	(42.7)		
Jejunum	51.4	46.5	48.4	47.3	49.8	47.0	0.44	0.71
weight, g	(3.2)	(2.8)	(3.7)	(3.4)	(2.5)	(2.4)		
Ovary weight,	11.32	12.34	12.05	11.73	-	-	-	0.97
g	(1.68)	(1.36)	(1.74)	(1.84)				
Testes weight,	804.7^{ab}	815.4^b	592.9^a	826.9^{ab}	-	-	-	0.26
g	(73.9)	(67.8)	(87.0)	(93.0)				

[1] F = female; M = male.
^{a,b} Values with a different superscript within a main effect (treatment or sex) differ by $P < 0.05$.

Least square means for organ weights are presented in Table 4.4 for treatment and sex main effects and in Figures 4.2 and 4.3 for treatment by sex interaction. There was no effect of treatment on pancreas, heart, heart fat, jejunum, and ovary weights ($P > 0.33$). While there was

not a significant treatment effect ($P = 0.26$), SB boars had significantly lighter testes than SUG boars ($P = 0.049$) and tended to have lighter testes than GB and CON boars ($P = 0.10$).

Additionally, SUG offspring had significantly heavier lungs than GB and CON offspring ($P = 0.006$ and 0.007, respectively) and tended to have heavier lungs than SB offspring ($P = 0.06$). There was no sex effect ($P > 0.18$) for pancreas, heart, lung, and jejunum weights. Boars had significantly heavier livers than gilts ($P = 0.005$). Gilts had more fat around their hearts based on weight than did boars ($P = 0.002$). There were treatment by sex interactions for both left kidney ($P = 0.07$; Figure 4.2) and left kidney fat ($P = 0.06$; Figure 4.3) weights. There was no difference between treatments for left kidney weight in gilts ($P > 0.37$). Boars from SB sows had significantly smaller left kidneys than boars from SUG or CON sows ($P = 0.006$ and 0.03, respectively). Additionally, SB boars did not differ in weight from SB gilts ($P = 0.17$) while GB, SUG, and CON boars had significantly larger kidneys than their respective gilts ($P < 0.002$).

Figure 4.2. Least square means for left kidney weight by treatment (supplementation of GB = ground beef, SUG = sugar, SB = combination of ground beef and sugar, and CON = corn-soy) for gilts (blue bars) and boars (orange bars). Bars with different letters (a,b,c) differ by $P < 0.05$.

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There were no significant differences between treatments in boars for left kidney fat weight ($P >$ 0.18). While there were also no significant differences between treatments in gilts, GB gilts tended to have more left kidney fat than SUG gilts ($P = 0.08$).
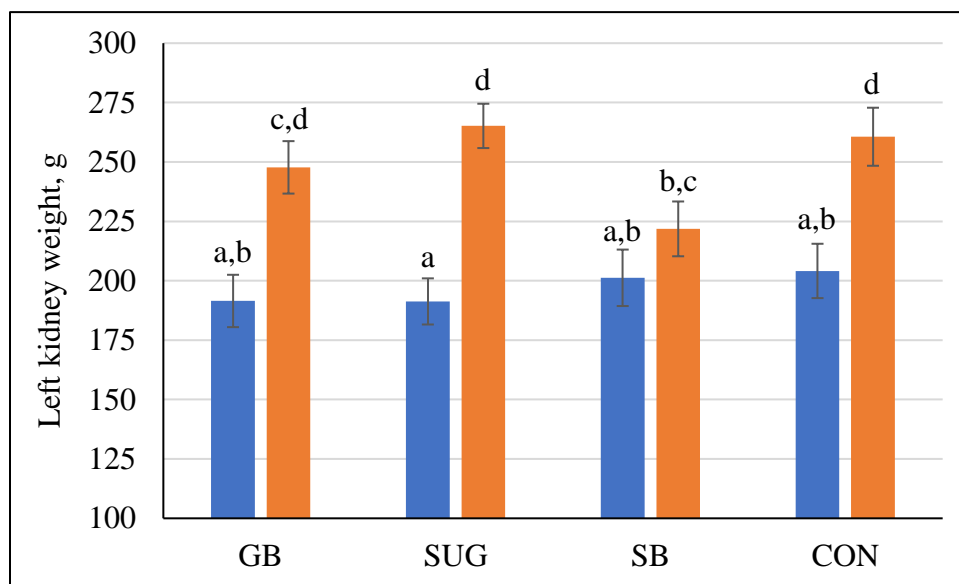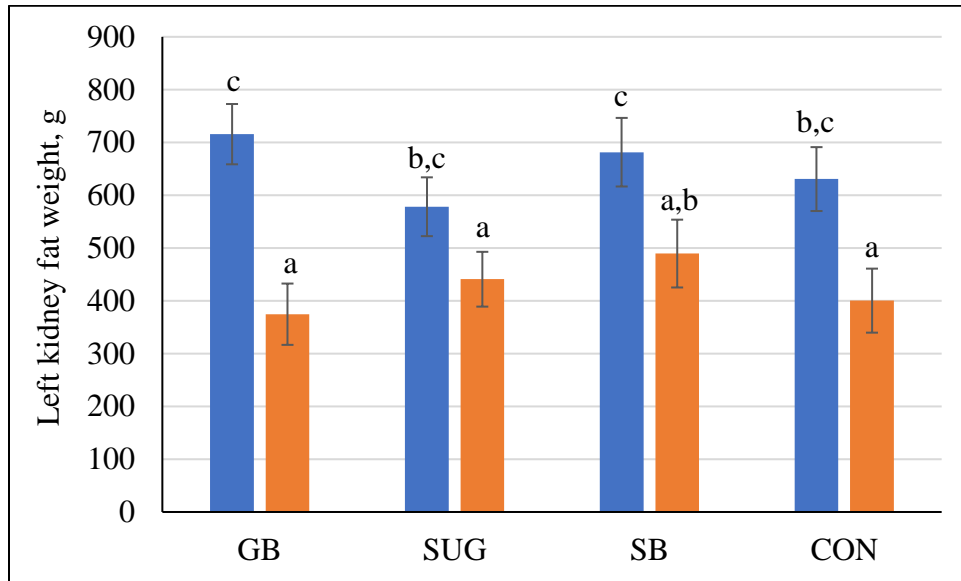

Figure 4.3. Least square means for left kidney fat weight by treatment (supplementation of GB = ground beef, SUG = sugar, SB = combination of ground beef and sugar, and CON = corn-soy) for gilts (blue bars) and boars (orange bars). Bars with different letters (a,b,c) differ by $P < 0.05$.

As shown in Table 4.5, there were no significant differences seen for sex or treatment on CIE L*, a*, and b* color scores measured on the liver, kidney, or longissimus dorsi chop ($P >$ 0.05). However, L* measured on the outside of the liver tended to be lower in gilts than boars (P = 0.098).

When examining the right longissimus dorsi muscle (**LD**) obtained from offspring, there was a sex by treatment interaction seen ($P = 0.03$; Figure 4.4). For CON offspring, gilts had heavier LD than boars (477.7 vs. 406.7 g, respectively; $P = 0.04$), with no other treatments differing between gilts and boars ($P > 0.1$). There was also a tendency for GB gilts to possess lighter LD when compared to CON gilts (403.6 vs. 477.7 g, respectively; $P = 0.05$). There were no significant differences between treatments for boars ($P > 0.19$).

Table 4.5. Least square means (standard error) by sex and treatment (**TRT**) of liver, kidney, and longissimus dorsi (**LD**) chop Minolta colors (L*, a*, and b*) for offspring whose mothers were fed cooked ground beef (**GB**), sugar (**SUG**), a combination of cooked ground beef and sugar (**SB**), or a control of corn-soy (**CON**) supplementation through gestation and lactation.

Trait[2]	Treatment				Sex[1]		P-value	
	GB	SUG	SB	CON	F	M	Sex	TRT
Liver								
L*	35.48	35.63	35.43	35.56	35.01	36.04	0.098	0.996
	(0.57)	(0.55)	(0.70)	(0.60)	(0.43)	(0.43)		
a*	10.80	11.31	10.97	11.33	10.84	11.36	0.12	0.42
	(0.26)	(0.25)	(0.32)	(0.28)	(0.22)	(0.21)		
b*	3.44	3.42	3.82	3.90	3.50	3.79		
	(0.49)	(0.47)	(0.60)	(0.52)	(0.40)	(0.40)		
Kidney								
L*	47.38	47.50	47.26	48.69	47.89	47.52	0.60	0.46
	(0.67)	(0.64)	(0.81)	(0.70)	(0.49)	(0.49)		
a*	13.13	13.08	12.89	12.28	13.05	12.65	0.32	0.50
	(0.43)	(0.41)	(0.53)	(0.45)	(0.30)	(0.30)		
b*	9.66	10.24	9.88	10.05	9.91	10.00	0.84	0.84
	(0.48)	(0.46)	(0.58)	(0.52)	(0.35)	(0.36)		
LD chop								
L*	44.57	44.72	45.82	44.47	44.33	45.46	0.13	0.60
	(0.66)	(0.62)	(0.82)	(0.68)	(0.52)	(0.49)		
a*	16.38	16.34	16.58	16.81	16.54	16.52	0.94	0.55
	(0.25)	(0.24)	(0.32)	(0.26)	(0.22)	(0.20)		
b*	3.44	3.54	3.62	3.54	3.47	3.60	0.65	0.98
	(0.26)	(0.24)	(0.32)	(0.26)	(0.21)	(0.19)		

[1] F = female; M = male.
[2] L* is a measure of lightness/darkness where 0 = pure black and 100 = pure white; a* is a measure of green/red where negative values are green and positive values are red; b* is a measure of blue/yellow where negative values are blue and positive values are yellow.

There were no treatment effect seen for the right semimembranosus with adductor weight or the right semitendinosus weight ($P = 0.94$ and 0.84, respectively; Table 4.6). While there was no difference in right semimembranosus with adductor weights between sexes ($P = 0.47$), the right semitendinosus was heavier in gilts than boars ($P = 0.02$). Additionally, there were no treatment or sex effects for LMA ($P = 0.80$ and 0.19, respectively; Table 4.6). When considering both subcutaneous (BF) and intramuscular fat (SMS) for the longissimus dorsi, there were no treatment effects seen ($P = 0.20$ and 0.60, respectively; Table 4.6). While there was no difference
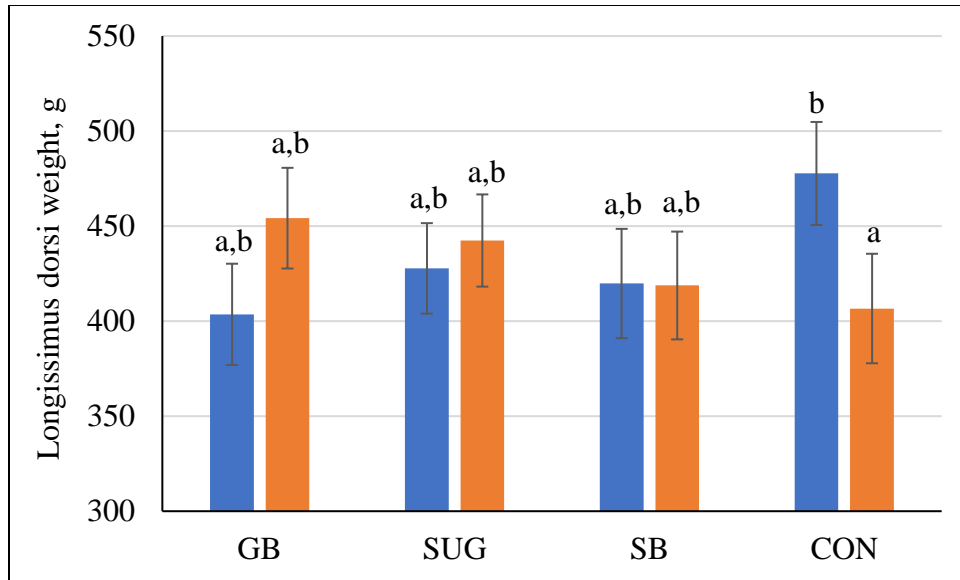
Figure 4.4. Least square means for right longissimus dorsi muscle weight by treatment (supplementation of GB = ground beef, SUG = sugar, SB = combination of ground beef and sugar, and CON = corn-soy) for gilts (blue bars) and boars (orange bars). Bars with different letters (a,b) differ by $P < 0.05$.

Table 4.6. Least square means (standard error) by sex and treatment (**TRT**) of the weight of the right longissimus dorsi (**RLD**) weight, right semimembranosus with adductor (**RSMA**) weight, right semitendinosus (**RST**) weight, longissimus dorsi muscle area (**LMA**), backfat (**BF**), and subjective marbling and color scores for offspring whose mothers were fed cooked ground beef (**GB**), sugar (**SUG**), a combination of cooked ground beef and sugar (**SB**), or a control of corn-soy (**CON**) supplementation through gestation and lactation.

| Trait | Treatment | | | | Sex | | P-value | |
	GB	SUG	SB	CON	F	M	Sex	TRT
RSMA weight, g	2125	2167	2101	2141	2163	2104	0.47	0.94
	(78)	(67)	(89)	(85)	(57)	(55)		
RST weight, g	628.0	650.0	636.7	643.9	663.6[a]	615.7[b]	0.02	0.84
	(18.6)	(16.7)	(21.7)	(19.86)	(13.8)	(13.7)		
LMA, cm^2	55.11	56.57	54.69	56.85	57.30	54.31	0.19	0.80
	(1.74)	(1.64)	(2.06)	(1.94)	(1.51)	(1.40)		
BF, cm	2.62	2.67	2.37	2.91	2.53	2.75	0.25	0.20
	(0.15)	(0.14)	(0.18)	(0.17)	(0.13)	(0.12)		
Subjective marbling score	1.23	1.16	1.32	1.22	1.33[a]	1.13[b]	0.006	0.60
	(0.08)	(0.07)	(0.09)	(0.08)	(0.05)	(0.05)		

[a,b] Values with a different superscript within a main effect (treatment or sex) differ by $P < 0.05$.
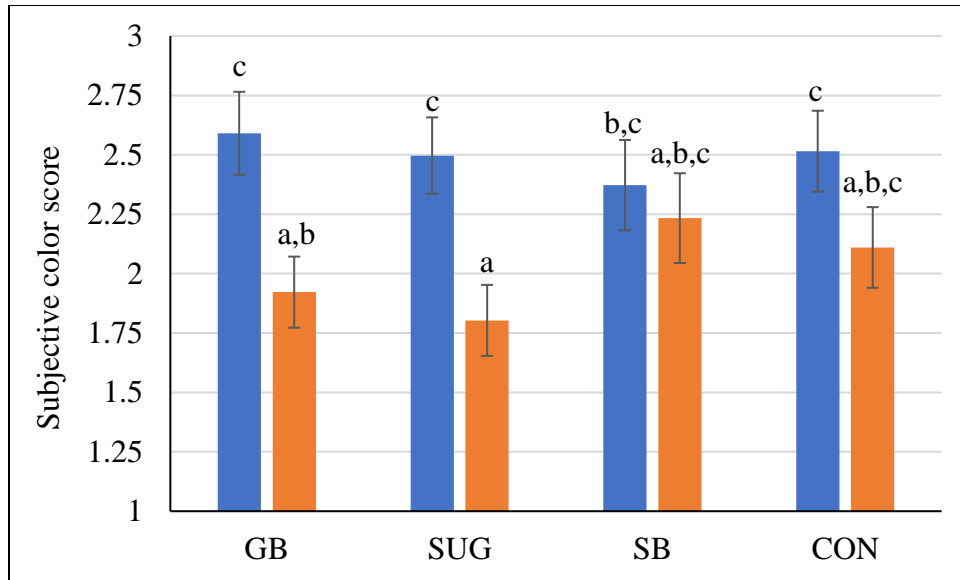
Figure 4.5. Least square means for subjective color score (1 = pale in color, 6 = dark in color) by treatment (supplementation of GB = ground beef, SUG = sugar, SB = combination of ground beef and sugar, and CON = corn-soy) for gilts (blue bars) and boars (orange bars). Bars with different letters (a,b,c) differ by $P < 0.05$.

between sexes for BF ($P = 0.25$), gilts had higher SMS than boars ($P = 0.006$; Table 4.7). There was a sex by treatment interaction for SCS ($P = 0.08$; Figure 4.5). While there were no differences between treatments in gilts ($P > 0.41$), SB boars tended to have higher SCS than SUG boars (2.23 vs. 1.80, respectively; $P = 0.08$). Additionally, both GB and SUG gilts had greater SCS than their respective boars ($P = 0.006$ and 0.002, respectively), with CON gilts only tending to have greater SCS than CON boars ($P = 0.098$). There was no difference seen in SCS between SB gilts and SB boars ($P = 0.61$).

Discussion

When examining offspring carcass composition, the weight differences between sexes can be due to the physical differences observed between gilts and boars. Ultimately, boars reach heavier weights sooner than that of barrows and gilts. Previous studies examining the effects of gender on carcass composition and quality have produced similar results with males having heavier PCW (Newell and Bowland, 1972; Schwab et al., 2007; Martin, 2008). When further

examining characteristics of boars and gilts, our findings contradicted that of other studies where boars deposited more backfat than gilts in this trial. In this present study there was no difference between sexes for tenth-rib backfat deposition measured post-rigor. While Schwab et al. (2007) found that tenth-rib backfat deposition rates showed a significant difference between boars and gilts with boars depositing backfat faster than gilts, resulting in gilts having the slowest deposition rate. While this present study showed there was no difference in longissimus muscle area, Beattie et al. (1999) has previously shown that when compared with boars, gilts had a greater longissimus dorsi area given a common age and body weight.

Perirenal fat has been associated with an adverse metabolic risk including poor blood pressure control and chronic kidney disease (Ma et al., 2015; Roever et al., 2015). The adipose tissue surrounding the kidney is not only a means for energy storage but also a metabolically active tissue, regulating the physiology and pathophysiology of adjacent organs (Ma et al. 2015). When adipose tissue reaches maximal expansion capacity, an "overflow" of lipids from adipocytes can occur (van Hees et al., 2010; Daemen et al., 2018). Lipids then begin to accumulate in various abnormal sites including, but not limited to, visceral adipose tissue, intrahepatic, intramuscular, and pericardial fat, a phenomenon leading to lipotoxicity (Gray and Vidal-Puig, 2007). In this study, gilts had significantly more peri-renal fat compared to the boars; however, there was no other abnormal lipid deposition observed in the present study comparing across treatments or sexes. This contradicts the findings of Einset et al. (2010) who examined the relationship between BMI, gender, and the distribution of subcutaneous and perirenal fat and found that women exceed men in subcutaneous fat, while men exceed women in perirenal fat.

When examining testicular weight differences, little research has been done on how the addition of excess sugar or protein to the maternal diet will affect the development of offspring

reproductive organs. However, previous studies found that excess maternal nutrition in domestic animals has been shown to affect testicular development and to delay the onset of puberty (Da Silva et al., 2001; Duppont et al, 2004; Weller et al., 2015; Robles et al., 2017). In this study, the higher testicular weights may be contributed to the greater fat accumulation that was seen around the testicles at the time of collection, as was also weighed with the testicles when the weight was collected.

Our findings in this study contrast to what would normally be expected regarding minolta color scores and fat ratio for boars and gilts used for meat production. In this study, the longissimus muscle color of gilts was darker in color when compared to the boars which contradicts other studies showing that boar meat presented lower values of L* (brightness) and b* (yellow color intensity), and higher values of a* (red color intensity). Additionally, it has been shown by Piao et al. (2004) that, as weight increases, so will L*, a* and b*. Piao et al. (2004) also showed that the a* value is usually greater in gilts but L* value and b* value is not affected by sex of pigs, which differs from our current study showing gilts have darker longissimus dorsi. Lastly, with no differences seen in color scores of the liver between treatments or sexes, we can conclude that there is no concern for non-alcoholic fatty liver disease because a "pale" liver may be an indication of a greater content of hepatic adipose.

When examining the IMF and color score of the offspring LD, the more IMF present in the LD for the gilts may be explained by gilts potentially consuming more feed during their time on test. It is interesting that the offspring boars did not have dark color scores. Usually heavier carcasses will cause dark colored longissimus dorsi muscles, however in this study we saw the opposite with gilts possessing dark subjective color scores. This may be due to higher stress or motility issue that were observed with the gilts prior to harvest. As animal handlers, ways to

alleviate stress prior to harvest is extremely important. All pigs had chance to rest when they come off the hauling truck at the NDSU meat laboratory. Also, it was ensured that all employees working with the pigs practiced proper handling procedures to reduce stress before harvest. The present data provides some interesting information to develop a study regarding maternal sugar intake and how sugar would affect placenta growth and the flow of nutrients across the placenta to alter testicular development.

Conclusion

The role of maternal diets in early offspring development has been studied extensively in both human and animal models. In this present study it can be concluded that the consumptions of a basal diet in addition to an added protein (meat) or sugar supplementation did not alter mature body composition between dietary treatments. Further examination of offspring growth curve will need to be performed to determine how maternal supplementation effects early offspring development. While there were sex differences seen, this was to be expected when comparing gilt versus boar physiological growth status. Moreover, there is potential to continue performing research on how added protein during pregnancy may alter the health of their offspring. In addition, studies should be performed on the effects of sugar and meat supplementation during early stages of development directly to the offspring and after maternal nutrition has been altered.

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APPENDIX A. COMPARISON OF FASTING VERSUS NON-FASTING

CHOLESTEROL CONCENTRATIONS USING A SWINE BIOMEDICAL MODEL

Materials and Methods

This study was conducted at the North Dakota State University (**NDSU**) Swine Research Unit (Fargo, ND). All animal care and handling procedures were approved by the Institutional Animal Care and Use Committee (protocol #A19030) under the supervision of the attending veterinarian.

Animals and Feeding Protocols

Fourteen sows of maternal genetics (Yorkshire or Yorkshire x Chester White) were selected from the herd at the NDSU Swine Research Unit. Three sows per day were bled over the duration of this study; once prior to their morning feed and then again 1-h post feeding. A total of 28 samples were collected, 2 samples per sow.

The day prior to blood being drawn, sows were monitored during their morning feeding. Standard feeding procedures for gestation sows are to be fed only once a day in a commercial production unless they are in pen housing with electronic feeders. The amount of feed as well as the time at which eating was finished was recorded. The following day, sows were bled prior to their morning feeding and after fasting (~24 h). This initial blood collection was for fasted blood levels in a lipid panel and triglyceride analysis. After the initial fasted blood draw was performed, sows were fed and monitored while eating, with both the start and finish time of eating being recorded. One-hour post the finish time of eating, sows were bled again for non-fasted levels and time was recorded.

Blood Collection Protocol

Sows were restrained manually whilst standing using a snout snare to safely secure the animal in their individual pens. This technique allows the head to be restrained and elevated parallel to the ground. The snout snare was positioned behind the canine teeth and the neck was lifted upward to better expose the jugular groove. Pigs were restrained for a maximum of 3 min and then released. To limit injury and bruising at the sampling site, no more than 3 attempts were made if bleeding was unsuccessful.

Blood was collected from the external jugular vein, 25 to 40 mm below the skin, using a 1.5" 20G needle and a vacutainer tube. The needle was inserted slightly to the mid line 120 mm above the point of the shoulder and was advanced at a 45° angle towards the left shoulder of the pig. The vessel was accessed from the right side of the thoracic inlet to avoid the vagus and recurrent laryngeal nerves, which are more prominent on the left side. The blood sample collected were used for lipid panel determination were collected in 15-mL standard glass blood collection tubes fitted with silicone-coated stoppers. Blood was allowed to clot in a cooler on wet ice for up to 1 h, then centrifuged (swinging bucket TS-5.1-500, 3000 x *g*, 20 min, 4 °C; Allegra 25R Centrifuge, Beckman Coulter, Fullerton, CA). Serum was aliquoted, frozen, and stored at -20 °C until analysis of insulin and a full lipid panel [total cholesterol (**TOTch**), high-density lipoprotein cholesterol (**HDLch**), and triglycerides (**TG**)].

Blood Chemistry Analysis

Serum TOTch was determined using the Infinity Cholesterol Liquid Stable Reagent (TR 13421; Thermo Fisher Scientific, Inc., Middletown, VA) and 200 mg/dL Stock Cholesterol Standard (C7509-STD, Pointe Scientific, Inc.). The assay was modified for a microtiter plate reader and used a sample-to-reagent ration of 1:50, with a total reagent volume of 250 µL. Serum

samples were incubated at 37 °C with a 10-min incubation time. Colorimetric endpoint assay was read at a wavelength of 500 nm.

Serum HDLch was determined using the Liquid autoHDL Cholesterol Reagent Set [Reagent 1 and Reagent 2] (H7545-80; Pointe Scientific, Inc., Canton MI) and 56 mg/dL auto HDL/LDL Cholesterol Calibrator (H7545-CAL; Pointe Scientific, Inc., Canton, MI). The assay was modified for a microtiter plate reader and used a sample-to-Reagent 1 ratio of 1:45 and sample-to-Reagent 2 ration of 1:15, with a total reagent volume of 300 µL with a total sample-to-total reagent ratio of 1:60. Serum samples were incubated at 37 °C in Reagent 1 for 10 min. After the first incubation, Reagent 2 was added, and the sample was incubated for an additional 10 min at 37 °C. Colorimetric endpoint assay was read at a wavelength of 600 nm.

Serum TG were determined using the Infinity Triglyceride Liquid Stable Reagent (TR22421; Thermo Fisher Scientific, Inc., Middletown, VA) and 200 mg/dL Glycerol Standard Solution (T7531-STD; Pointe Scientific, Inc., Canton MI). The assay was modified for a microtiter plate reader and used a sample-to-reagent ration of 1:50, with a total reagent volume of 250 µL. Serum samples were incubated at 37 °C with an 8-min incubation at 37 °C. Colorimetric endpoint assay was read at a wavelength of 500 nm.

Statistical Analysis

Analyses were conducted using the mixed procedure in SAS (v. 9.4, SAS Institute, Cary, NC). The model included fixed effects of status being fasted or fed. A repeated measures statement with sow as the subject was fit in the model. Different covariate structures were tested and the best fit based on the Akaike and Bayesian Information Criteria was chosen. Least square means were calculated.

Results and Discussion

There were no significant differences seen in fasting versus fed circulating TG levels or HDLch levels (P = 0.25 and 0.24, respectively). However, when examining circulating TOTch levels, there was a difference seen in fasted levels having a greater circulating serum concentration of TOTch when compared to the TOTch in the fed state (57.8 vs. 53.4 mg/dL; P = 0.05). According to the serum biochemical reference ranges as noted by Fielder (2018), normal ranges of TOTch for swine range from 36-54 mg/dL. Considering the levels of TOTch found in this study, the fasted levels may be considered boarder high and would benefit from examine the cholesterol ratio and other levels to determine is these results are to be of any concern.

As previously mentioned, measurement of lipids has traditionally been recommended to be when the patient is fasting. With current guidelines, low-density lipoprotein (LDLch) cholesterol values may not be needed for some clinical scenarios (American College of Cardiology Foundation, 2019) For several risk estimators, LDLch is not included, but rather total cholesterol and high-density lipoprotein (HDLch) cholesterol, both of which vary little between the fasting and non-fasting state (Driver et al., 2016; American College of Cardiology Foundation,2019). Therefore, in the estimation of initial risk among the primary prevention patients who are not on lipid-lowering therapy, non-fasting lipid measurements would be acceptable. Additionally, the assessment of metabolic parameters which define metabolic syndrome can assist the provider and patient to initiate changes, in particular lifestyle changes, which reduce risk of diabetes and cardiovascular disease. Ideally, measurement of lipids (a criterion of the metabolic syndrome) is recommended to be measured in the fasting state. However, non-fasting measures including triglycerides >200 mg/dl and a low HDL (40 mg/dl or <50 mg/dl), would be consistent with traditional metabolic criteria and thus allow for

therapeutic interventions to begin soon after results are measured (Driver et al., 2016). Thus, for the assessment of metabolic syndrome, non-fasting would be acceptable.

When comparing the fasted vs. non-fasted levels of TOTch, this study showed that from a pathological stand point, there is little reason for concern in receiving TOTch levels from subjects in the fed state. Prospective studies in humans have shown that non-fasting lipids are similar to fasting lipids or better for predicting cardiovascular disease among groups of individuals (Bansal et al., 2007; Kolovou et al., 2011; Nordestgaard and Freiberg, 2011; Steriner et al., 2011; Rahman et al., 2018). This study demonstrated that in a swine biomedical model, the predicted values of lipids measured both fasting and non-fasting from the same individuals, showed little to no difference in TOTch, TG, and HDLch levels for individuals in the fasted or fed state. Furthermore, from this study we found that there is no significant clinical difference between fasting and non-fasting levels of TOTch, TG, and HDLch. Thus, this study suggests that non-fasting tests could be used to follow up dyslipidemic patients.

Conclusion

In this study we found that there was no significant clinical difference between fasting and non-fasting levels of TOTch, HDLch, and TGs. While LDLch specifically was unable to be analyzed in this present study, it is important for future research to examine how fasted or non-fasted blood samples could affect circulating LDLch Levels. Additionally, for future research calculating a cholesterol ratio may also be beneficial in determining risk factors for dyslipidemia or coronary heart disease.

Thus, there is a potential for practitioners and biomedical researchers to use the non-fasting method tests to follow-up with dyslipidemic patients. While some fasting lipids tests will remain necessary, particularly for people with very high triglycerides, fasting may not be

required for most, including those having routine cholesterol tests to weigh cardiovascular risk. Furthermore, this present study may also contribute to the clinical reliability of non-fasting determination of serum lipids for dyslipidemia diagnosis and coronary heart disease risk assessment in the future.

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