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The Investigation of Pregnancy Toxemia Diagnostics in Dairy Goats

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A thesis
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ABSTRACT

Pregnancy Toxemia is a common and widespread disease which prominently effects late pregnant goats and sheep. There is a lack of research and information on pregnancy toxemia in goats and the use of biomarkers and meters to measure pertinent blood values which can help diagnose the disease. The study objectives were to evaluate the accuracy of the CentriVet Handheld Blood Glucose and Ketone Monitoring Meter in measuring blood glucose and blood beta-hydroxybutyrate (BHB) values in dairy goats and to determine relevant blood changes and biomarkers in relation to a diagnosis of pregnancy toxemia. There was a high association between glucose values measured by the CentriVet handheld meter and the laboratory values ($r^2 = 0.852$, $P < 0.001$). Additionally, the BHB values measured by the CentriVet handheld meter had a high association with the laboratory values ($r^2 = 0.877$, $P < 0.001$). The CentriVet Handheld Blood Glucose and Ketone Monitoring Meter was validated for its use in measuring BHB and glucose in does at a farm level.

BHB and NEFA had a significant relationship ($P < 0.05$) demonstrating how non-esterified fatty acids (NEFA) mobilization of body fat is strongly related to ketone body synthesis. Additionally, a highly significant relationship was revealed between BHB and calculated anion gap as well as the relationship between BHB and total CO_2 ($P < 0.0001$). This relationship supports the idea that acid base balance is disrupted in does with pregnancy toxemia. NEFA and glucose did not have a significant relationship ($P > 0.05$) supporting the idea that glucose homeostasis disruption may not be a required component to the disease process. Other significant relationships were found during analysis which should be further explored moving forward.

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Chapter 1

Introduction

Pregnancy toxemia is a prevalent disease within late pregnant dairy goat populations. The disease can lead to substantial animal and production losses due to pregnancy toxemia's high mortality rate and the contribution of delayed treatment (Doré et al., 2015). Producers oftentimes fail to identify early clinical signs of the disease which can result in later treatment being unsuccessful due to the disease progression. Additionally, many goat producers resist veterinary service due to the cost of veterinary care, which also contributes to the disease mortality rates. The use of handheld meters to measure glucose and beta-hydroxybutyrate concentrations, two key blood values altered by pregnancy toxemia, can serve as a simple, rapid, and inexpensive approach to pregnancy toxemia diagnosis. This simple field-based tool would benefit producers as well as veterinarians. Additionally, there has been minimal research into pregnancy toxemia in goats as a whole and into other blood value changes that may occur in the late pregnant goats.

The study had two overall objectives. The first objective of the study was to validate the CentriVet Blood Glucose and Ketone Monitoring Meter by comparing meter whole blood values with laboratory serum analysis. The null hypothesis tested for the first objective was that the meter does not accurately quantify whole blood glucose or whole blood beta hydroxybutyrate in late pregnant does. The second objective of the study was to determine if metabolic parameters exist which may be used to better quantify pregnancy toxemia outcomes. The null hypothesis tested for the second objective was that no other parameter other than glucose or beta hydroxybutyrate can provide information on pregnancy toxemia.

Chapter 2

Literature Review

2.1 Introduction

Pregnancy toxemia is a common metabolic disease faced by late pregnant does, which can be fatal and result in substantial production losses. Pregnant does are put under a high level of metabolic stress that can be heightened by nutritional imbalances leading to hypoglycemia and elevated beta-hydroxybutyrate blood concentrations. Pregnancy toxemia prevalence may range from 5 to 20% amidst an outbreak (Rook, 2000). Doré and colleagues reported 10% of pregnant does identified with a high overall pregnancy toxemia risk in a commercial dairy (Doré et al., 2015). In this same study, an overall 5.5% mortality rate was observed from prepartum to one week postpartum (Doré et al., 2015). Mortality rates can exceed 80% of pregnancy toxemia affected does, especially with delayed treatment (Rook, 2000). Preventative measures and proper disease management can result in better doe and kid outcomes and less production losses. The purpose of this review is to discuss the pathogenesis of pregnancy toxemia in goats while also addressing the diagnostic, therapeutic, and preventative measures concerning the disease. Newer technologies allowing for earlier detection will be addressed.

2.2 Pathogenesis

2.2.1 Defining Pregnancy Toxemia

Pregnancy toxemia, also referred to as twin lamb or kid disease or ketosis, is a metabolic disease affecting does in late gestation, often within the last month of pregnancy and with pregnancies of two or more fetuses (Rook, 2000; Brozos et al., 2011). Additionally, the disease has greater prevalence in older does undergoing their second or greater pregnancy and less common in first time pregnant does (Rook, 2000). Metabolically, the disease is associated with reduced energy intake coupled with increased energy demand to support the fetuses, along with an abnormal metabolism of carbohydrates and fats, resulting in a state of negative energy balance (Rook, 2000; Brozos et al., 2011).

The disease can affect a range of pregnant does, with two common nutritional situations that result in pregnancy toxemia (Mongini and Van Saun, 2023). Nutritional situations of obesity or starvation can predispose the doe to pregnancy toxemia (Mongini and Van Saun, 2023). Obesity-related pregnancy toxemia often results from a doe's high body condition followed by a period of reduced intake and nutritional decline during late pregnancy (Rook, 2000; Mongini and Van Saun, 2023). Body condition scoring (BCS), on a scale of 1 to 5, can be used to determine the risk for the disease (Mongini and Van Saun, 2023). Does with a BCS of 4 or greater have an increased risk of pregnancy toxemia, hepatic lipidosis, and hyperglycemia (Brozos et al., 2011; Mongini and Van Saun, 2023). Additionally, obese does with excess abdominal fat have less rumen capacity and have a greater risk of developing lactational ketosis after birthing (Rook, 2000). On the other end of the spectrum, starved does face other risks. Starved does, with a BCS of 1 or lower, frequently face hypoglycemia and hypocalcemia due to under conditioning or

inadequate nutrition and intake (Mongini and Van Saun, 2023). A rapid health decline is common with starved does and ewes which can result in high death losses (Mongini and Van Saun, 2023).

2.2.2 Etiology

Pregnancy toxemia is related to glucose demand and balance between fetus and pregnant doe (Marteniuk and Herdt, 1988). The pregnant doe must allocate a large amount of glucose, approximately 30 to 40% of its maternal glucose supply, to the fetus who relies on mostly glucose, along with small amounts of lactate and amino acids, for its energy needs (Marteniuk and Herdt, 1988). The fetus' glucose demand may remain satisfied even at the expense of the dam's declining levels of glucose production (Rook, 2000). Within the last 6 weeks of gestation, the fetus grows by roughly 80%, which demands more glucose from the pregnant doe (East, 1983).

Several different causes can result in the pregnant doe experiencing negative energy balance. Inadequate nutrition along with reduced feed intake, usually due to improper diet formulation or decreased rumen capacity from pregnancy, are prevalent sources of negative energy balance and pregnancy toxemia (Ji et al., 2023; Mongini and Van Saun, 2023). Hypocalcemia can also be a precursor to negative energy balance and is usually associated with inadequate dietary calcium or reduced intake (Mongini and Van Saun, 2023). An inadequate water source or a lack of access to water can also prompt negative energy balance because water consumption is essential for dry matter intake and can therefore prompt a lack of intake (Mongini and Van Saun, 2023). Additionally, changes within the doe's feeding environment or

within their management can increase stress levels and contribute to reduced intake and intestinal microbiome alterations, making the dam more susceptible to diseases (Ji et al., 2023). As discussed with the two main types of does affected by pregnancy toxemia, poor dam mobility due to obesity or a lameness can hinder the doe's ability to eat and promote negative energy balance (Mongini and Van Saun, 2023).

A factor that may influence dam susceptibility to negative energy balance and pregnancy toxemia is insulin resistance (Rook, 2000). Insulin resistance may interfere with the dam's glucose regulation as the dam is unable to respond to insulin and therefore can fail to allocate sufficient glucose to the fetus (Rook, 2000). Insulin resistance can lead to increased fat mobilization to provide more energy and subsequent accumulation of non-esterified fatty acids (NEFAs) in the liver leading to fatty liver, a common accompaniment to pregnancy toxemia (Xue et al., 2019). Additionally, hepatic gluconeogenic response and the doe's genetics can also influence susceptibility to pregnancy toxemia (Marteniuk and Herdt, 1988; Ji et al., 2023).

During periods of negative energy balance, the pregnant doe may mobilize their body protein, which can be detrimental to the doe so there are adaptive mechanisms in place that allow for mobilization of energy reserves from fats (Herdt, 2000). Ketone bodies, compounds produced from fatty acids in the liver, which serve as a fuel source, play an important role in the dam's adaption to negative energy balance (Herdt, 2000). When a pregnant doe is undergoing negative energy balance, the use of NEFAs and ketone bodies for energetic purposes increases to reduce glucose use to help maintain blood glucose concentration (Herdt, 2000). When glucose and glucose precursors are low, a reduced amount of glucose enters the Krebs cycle which causes a reduction in the amount of citrate production for malonyl CoA production (Herdt, 2000). Decreased amounts of malonyl CoA activates carnitine palmityl transferase I (CPT I), an enzyme

necessary for NEFA transport for ketone body synthesis (Herdt, 2000). CPT I rapidly increases transport of NEFAs into the mitochondria where the mitochondria then metabolizes NEFAs, stimulating the production ketone bodies (Chow and Jesse, 1992; Herdt, 2000). All ketone bodies are derived from acetoacetate (Herdt, 2000). Both acetoacetate and acetone are unstable and not easily measured in body fluids. As such, beta-hydroxybutyrate (BHB) is the predominate ketone body measured in blood (Chow and Jesse, 1992; Herdt, 2000). Additionally, glycogen, which is stored in the liver when there is excess glucose in circulation, is a small source of glucose mobilization during a negative energy balance, but depletes quickly (Herdt, 2000). When these metabolic adaptations fail within the pregnant doe, pregnancy toxemia may develop (Herdt, 2000).

Ketosis may develop within a pregnant or early lactation animal recently pregnant animal due to a lack of gluconeogenesis substrates leading to a greater glucose demand than the amount produced via gluconeogenesis, resulting in a high concentration of ketone bodies (Herdt, 2000). Ketosis may also develop due to a high level of NEFA accumulation in the liver, leading to fatty liver, accompanied by moderate rates of ketogenesis and gluconeogenesis (Herdt, 2000).

Pregnancy toxemia is frequently associated with hypoglycemia and hyperketonemia (Mongini and Van Saun, 2023). When the pregnant doe is mobilizing their body fats during gluconeogenesis, there are increased levels of NEFAs within circulation, which stimulates uptake of fatty acids from the liver and the production of ketone bodies (Schlumbohm and Harmeyer, 2004). An increased concentration of ketone bodies within circulation results in a decrease in the hepatic rate of glucose production, furthering the progression of pregnancy toxemia (Schlumbohm and Harmeyer, 2004). Additionally, increased mobilization of body

protein may overwhelm the liver and lead to excess ketone body production (Vasava et al., 2016).

2.2.3 Disease Progression

Does experiencing pregnancy toxemia will undergo several prevalent clinical signs and changes in behavior as the disease progresses. Pregnancy toxemia typically progresses over the course of 3-10 days from the onset of initial clinical signs to the death of the pregnant doe if left untreated (Rook, 2000). During initial disease stages, the affected dam may separate herself from the rest of the flock and may appear disoriented (Marteniuk and Herdt, 1988). A decreased appetite is common in initial stages and affected animals may be seen approaching feeders with the rest of the flock but failing to eat (East, 1983; Marteniuk and Herdt, 1988; Rook, 2000). Pregnancy toxemia often goes undiagnosed and undetected during the onset of the disease, frequently resulting in disease progression prior to diagnosis (Marteniuk and Herdt, 1988; Rook, 2000).

After initial clinical signs, if left untreated the disease will progress as the pregnant doe shows a greater degree of depression and exhibits a range of neurologic clinical signs (Marteniuk and Herdt, 1988; Rook, 2000). The affected ewe or doe may present with neurologic signs such as teeth grinding, jaw clamping, impaired vision, lip twitching, convulsions, head pressing, muscle tremors, increased signs of disorientation, and exhibit a star-gazing head posture (East, 1983; Marteniuk and Herdt, 1988; Rook, 2000). The affected ewe or doe may also exhibit constipation and anorexia along with increased weakness and mental dullness during the later stages of the disease (Rook, 2000). Recumbency and the doe's inability to stand is a sign of a

poor prognosis and will occur 3-4 days after the onset of the initial clinical signs if left untreated (Rook, 2000). Following the 3-4 days of initial clinical signs, the affected animal will be unable to rise and will undergo initially sternal recumbency and advance to lateral recumbency (Marteniuk and Herdt, 1988). Without treatment the affected animal will become comatose and die after another 3-4 days following recumbency (Marteniuk and Herdt, 1988; Rook, 2000). If the fetus dies within the pregnant doe affected by pregnancy toxemia, the doe may recover from the disease spontaneously (East, 1983; Marteniuk and Herdt, 1988). However, prior to the death of the fetus, the fetus may become infected by bacteria which can result in dystocia and potential doe septicemia (Marteniuk and Herdt, 1988; Rook, 2000).

2.3 Diagnosis

2.3.1 Physical Assessment

Physical examination can serve as a preliminary diagnostic method to diagnose pregnancy toxemia. The doe affected by pregnancy toxemia will typically present with poor body condition due to inadequate nutrition to meet demand (Mongini and Van Saun, 2023). Typical clinical signs can be used as disease indicators with special attention to reduced mobility, decreased appetite, and depression (Mongini and Van Saun, 2023). These clinical signs are common for affected animals and can worsen if the condition remains untreated (Rook, 2000; Mongini and Van Saun, 2023). During initial disease stages, disease detection is challenging but physical examination may reveal early stages of neurologic dysfunction including an absent eye-preservation reflex, decreased pupillary light reflex, normal rumen motility, and a normal temperature, pulse, and respiratory rate (Marteniuk and Herdt, 1988).

2.3.2 Blood Testing

Analyzing the pregnant doe's blood can be used as a diagnostic measure for pregnancy toxemia. An increased concentration of blood beta-hydroxybutyrate (BHB) can be a conformational diagnostic blood test for pregnancy toxemia as the concentration is indicative of hyperketonemia (Bani Ismail et al., 2008; Mongini and Van Saun, 2023). A BHB blood concentration of 0.86 mmol/L or more is indicative of subclinical pregnancy toxemia (Bani Ismail et al., 2008). Other researchers have validated a 0.8 mmol/L BHB threshold to predict subclinical pregnancy toxemia (Doré et al., 2013). Although BHB is an efficient indicator of hyperketonemia, urine ketone strips can also serve as a diagnostic tool for hyperketonemia with a positive reaction (Rook, 2000). In pregnant does with subclinical pregnancy toxemia, a significant linear correlation has been found between the doe's BHB concentration and glucose concentration (Bani Ismail et al., 2008). In addition to elevated BHB concentrations, pregnant does with subclinical pregnancy toxemia are also more likely to also have hypoglycemia, azotemia, and hyperproteinemia (Bani Ismail et al., 2008).

Other blood tests can serve as diagnostic aids for diagnosing pregnancy toxemia. Myocardial damage occurring from pregnancy toxemia can be detected from elevated values of cTnI and CK-MB and can serve as an indicator of pregnancy toxemia for goats (De Souza et al., 2020). Pregnancy toxemia has been characterized by decreased levels of calcium and glucose along with increased levels of Serum Glutamic Pyruvic Transaminase (SGPT), Serum Glutamic-Oxaloacetic Transaminase (SGOT), Blood Urea Nitrogen (BUN), creatinine, BHB, and NEFA (Vasava et al., 2016).

2.3.3 Diagnostic Technology

With technology advances, more tools have been created and validated to allow for faster and cost-effective pregnancy toxemia diagnoses. Hand-held meters, such as the Precision Xtra (Abbott Industries, Chicago, IL), can be used to measure prognostic indicators of pregnancy toxemia in dairy goats including BHB with a high level of accuracy compared to standard testing (Doré et al., 2013). Ketonemia can therefore be monitored in dairy goats and can be used to indicate a worsening pregnancy toxemia condition with accuracy (Doré et al., 2013). Other hand-held devices including the FreeStyle Precision (Abbott Industries, Europe) and GlucoMen LX Plus (A. Menarini Diagnostics, Florence, Italy) were also tested for their capability to accurately measure BHB in dairy goats and the devices showed moderate to strong correlations with the gold standards (Pichler et al., 2014a). The Precision Xceed meter (Abbott Diabetes Care Inc., Alameda, CA) is another hand-held meter validated which has significant correlations between glucose and BHBA concentrations, serving as a diagnostic tool for pregnancy toxemia (Panousis et al., 2012). Validation and use of hand-held meters could allow for a less expensive and on-site diagnosis of pregnancy toxemia in goats.

2.4 Treatment

2.4.1 Nutritional Changes

After a pregnant doe has been diagnosed with pregnancy toxemia, nutrition should be analyzed to determine potential causes of the negative energy balance so diet and treatment methods can be initiated (Mongini and Van Saun, 2023). Treatment usually includes increasing

the affected animal's energy and glucose supply and treating secondary issues such as dehydration (Marteniuk and Herdt, 1988). Depending on the animal's state and how much the disease has progressed, especially if the ewe or doe is already recumbent, treatment options may be unsuccessful (Marteniuk and Herdt, 1988).

2.4.2 Medical Intervention

The administration of energy sources is a primary form of medical care for animal's experiencing pregnancy toxemia (Brozos et al., 2011). A common form of treatment is having an intravenous catheter placed in the affected animal to administer glucose (5-7 grams) every 3-4 hours until clinical signs improve (Rook, 2000). Propylene glycol and glycerol are two common glucogenic supplements used for pregnancy toxemia treatment in does (Marteniuk and Herdt, 1988; Alon et al., 2020). Upon analysis after drenching, propylene glycol is efficient at reducing BHB concentration whereas glycerol functions to increase glucose concentrations in does (Alon et al., 2020).

Removal of the fetuses through medical intervention is a form of treatment which may improve the health of the affected ewe or doe (Rook, 2000). Fetuses can be removed using a prostaglandin-induced parturition or through cesarean section and may allow for the recovery of the doe as their energy requirements decrease without the fetus (Rook, 2000; Brozos et al., 2011). Leading up to the final days of gestation, dairy goats exhibited an increase in glucose, which has been theorized to be explained by the death of the fetuses (Lima et al., 2012). Euthanasia is another consideration, if other medical and nutritional interventions have been exhausted or if the dam's condition has progressed to the terminal stages (Brozos et al., 2011).

2.5 Prevention

2.5.1 Diagnostic tools

Hand-held meters can also be used for prevention of pregnancy toxemia. A BHB concentration greater than 1.1 mmol/L, as measured by a hand-held meter, places pregnant ewes at a greater risk for hyperketonemia (Pichler et al., 2014b). Hand-held meters can be used to test a pregnant doe for their BHB concentration to determine if they are at a greater risk for pregnancy toxemia and management can then adjust to prevent the onset of the disease (Pichler et al., 2014b). Meters can therefore be used to analyze the doe's BHB and glucose concentration to diagnose pregnancy toxemia more easily.

2.5.2 Management Changes

Following a case of pregnancy toxemia or an outbreak within a herd, management changes should follow to prevent more cases. Considerations and changes should be made for does recovering from pregnancy toxemia because of their greater chance of relapsing the disease (Mongini and Van Saun, 2023). Does likely to relapse with pregnancy toxemia have an increased age, have structural unsoundness, or have a higher fetal kid weight (Mongini and Van Saun, 2023). These does with an increased risk should be monitored carefully with regular ultrasounds and exams to monitor the number of kids (Mongini and Van Saun, 2023). All does should be carefully monitored in late gestation to ensure that if a doe is losing weight, they can receive nutritional supplementation (Marteniuk and Herdt, 1988). Additionally, first time pregnant does should not be grouped with mature does (Mongini and Van Saun, 2023).

2.5.3 Nutrition

A balanced diet including proper rations can prevent pregnancy toxemia cases which result from an inadequate energy source or nutrition (Marteniuk and Herdt, 1988; Mongini and Van Saun, 2023). In flocks and herds which have undergone a recent pregnancy toxemia outbreak, ration energy content should increase for the final four weeks of pregnancy (Mongini and Van Saun, 2023). However, leading up to breeding, does should not be overfed because obesity can increase the likelihood of pregnancy toxemia (Marteniuk and Herdt, 1988). It is common for pregnant does to decrease their dry matter intake in late pregnancy so the diet should be modified to allow the pregnant doe to still receive adequate nutrition (Mongini and Van Saun, 2023). Elevating the amount of grain in late pregnancy diets can supplement the diet and aid does in receiving proper nutrients when facing low energy availability and can aid in microbial growth and protein production (Marteniuk and Herdt, 1988; Mongini and Van Saun, 2023). Since inadequate protein content in diets can be a major factor leading to pregnancy toxemia, the diet should be formulated to meet protein needs (Mongini and Van Saun, 2023).

2.6 Conclusion

Pregnancy toxemia continues to be a prevalent disease among late pregnant does around the world. Given the quick progression of the disease and subclinical presentation that often goes undetected, prevention methods are crucial to protecting ewes and does from the disease. More research should be conducted to further understand the pathogenesis of pregnancy toxemia in does and to develop more prevention techniques. Hand-held meters are a novel research focus

and should be further explored to determine their validity in diagnosing and preventing pregnancy toxemia.

Chapter 3

Validation of the CentriVet Blood Glucose and Ketone Monitoring Meter

3.1 Introduction

Pregnancy toxemia is a common and widespread metabolic disease facing late pregnant does which can be fatal and result in substantial production losses. Mortality rate can exceed 80% of pregnancy toxemia affected does, especially with delayed treatment (Rook, 2000). Metabolically, the disease is associated with reduced energy intake coupled with increased energy demand to support the fetuses, along with an abnormal metabolism of carbohydrates and fats, resulting in a state of negative energy balance (Rook, 2000; Brozos et al., 2011). When a pregnant doe is undergoing a negative energy balance, the use of NEFAs and ketone bodies for energetic purposes increases to reduce glucose use to help maintain blood glucose concentration (Herdt, 2000). An increased concentration of ketone bodies within circulation results in a decrease in the hepatic rate of glucose production (Schlumbohm and Harmeyer, 2004). Additionally, increased mobilization of body protein may overwhelm the liver and lead to excess ketone body production and further the disease progression (Vasava et al., 2016).

Preventative measures and proper disease management can result in better doe and kid outcomes and less production losses. Physical Assessment can be used as a preliminary diagnostic tool for the disease. The doe affected by pregnancy toxemia will typically present with poor body condition due to inadequate nutrition to meet demand (Mongini and Van Saun, 2023). However, clinical signs can be non-specific and can be challenging to diagnose before the disease progresses even more, negatively impacting the doe. Additionally, blood samples can be collected and sent to a laboratory for testing but there are negatives to this diagnostic method due

to the overall cost and the time it takes to produce test results which can delay medical intervention for the doe.

The ability for on-farm technology to accurately measuring blood glucose and BHB values could allow for a fast, more accessible, and less expensive tool for diagnosing pregnancy toxemia in does. It can be expensive for producers to receive veterinary care on-farm so the tool could minimize the labor and cost of measuring pertinent blood values in relation to the disease. Additionally, pregnancy toxemia may first present with a subclinical form of the disease and can progress with eventual clinical signs. A BHB value of 0.86 mmol/L or more has been identified as an indication of subclinical pregnancy toxemia and the use of meters could allow for producers to measure blood values and help diagnose the disease sub clinically (Bani Ismail et al., 2008). Additionally, BHB and glucose values can be monitored in dairy goats using the meter and can be used to indicate a worsening pregnancy toxemia condition overtime (Doré et al., 2013).

The use of handheld meters to diagnose pregnancy toxemia has been explored in dairy sheep and dairy cows. The Precision Xtra, a handheld meter, was validated for its prediction of BHB values in dairy cows when compared to laboratory testing with an R^2 of 0.98 (Pineda and Cardoso, 2015). In the study, the Precision Xtra was found to accurately discriminate between samples with a BHB value less than 1.2 mmol/L and samples with a BHB value greater than or equal to 1.2 mmol/L, the threshold level for pregnancy toxemia diagnosis in dairy cows (Pineda and Cardoso, 2015). In another study, the BHBCheck meter, was validated as accurately determining the whole blood, plasma, and serum BHB in cows with sensitivity (Suthar and Patil, 2021). A third meter, the Precision Xceed meter was evaluated for its use in measuring BHB and

glucose in late pregnant dairy sheep (Panousis et al., 2012). The meter had a strong significant correlation to laboratory testing for BHB with an R^2 of 0.99 but it had a weaker correlation with glucose with an R^2 of 0.76 (Panousis et al., 2012). This trend of a weaker correlation with glucose versus that of BHB has appeared in other studies for dairy cows (Panousis et al., 2012).

The CentriVet Blood Glucose and Ketone Monitoring Meter is calibrated to have a narrower scale for BHB at the lower end of the BHB concentration spectrum. We hypothesize that we can accurately measure doe glucose and BHB concentrations with the CentriVet handheld meter. By validating the CentriVet Blood Glucose and Ketone Monitoring Meter, a new diagnostic tool for pregnancy toxemia can be incorporated into farms. The validation of the handheld CentriVet Blood Glucose and Ketone Monitoring Meter in accurately measuring blood glucose and BHB in late pregnant does is the focus of this portion of the research study. This paper will discuss the materials and methods, results, discussion, and conclusions of the research project.

3.2 Materials and Methods

All procedures were reviewed and approved by the Pennsylvania State University Institutional Animal Care and Use Committee (IACUC#: PRAMS201747925).

3.2.1 Sample Collection

Whole blood was collected into blood tubes containing no anticoagulants or additives. Whole blood was collected from late pregnant does among 7 farms over a period between 2018

and 2023. Sampled does were any animal in the last month of pregnancy irrespective of presentation of clinical signs associated with pregnancy toxemia. Farm veterinarians or veterinary technicians collected whole blood samples for immediate testing and harvesting of serum. All samples collected were organized within a spreadsheet along with the date collected and with any relevant doe information including doe birth date, date kidded and sampled, doe health history, doe survival, kid number born, gender, and their mortality or survivability.

3.2.2 Sample Analysis

Collected whole blood samples were immediately tested on-farm by the herd veterinarian or supporting technicians for glucose (mg/dL) and BHB (mmol/L) concentrations using the provided CentriVet hand-held meter (https://www.aconlabs.com/brands/centrivet/gk-blood-glucose-ketone-monitoring-system/). Test strips used were those marketed for bovine diagnostics for glucose and BHB concentrations. All participating farms were provided instructions via a video on how to properly use the testing meter and test strips. Testing results were recorded in the provided data spreadsheet for each of the farms. The blood sample was then either processed on the farm to harvest serum to send to our laboratory or directly returned to our laboratory for processing. Participating farms not local to Penn State University were provided a centrifuge for serum harvesting as needed. All harvested serum samples were catalogued and frozen (-80°C) until sent for analysis. Catalogued serum samples were batched and sent to the Oregon State Veterinary Diagnostic Laboratory, a commercial laboratory, to perform their metabolic profile clinical chemistry analysis by which we focused on glucose and BHB laboratory results.

3.2.3 Statistical Analysis

All measures were evaluated for normality using Proc Univariate (SAS ver. 9.4, Cary, NC). Measured parameters found not to be normally distributed were transformed using either natural logarithm or reciprocal functions. Population demographics were determined by the Proc Means procedure. Spearman correlation coefficients were determined for all measured parameters. Linear regression (Proc Reg) was used to determine the relationship between whole blood meter determinations of glucose and BHB concentrations compared to laboratory determination in serum.

3.3 Results

Table 1. Sample Collection Breakdown

Farm Number	**Sample Collector**	**Number of Samples**
1	Herd Veterinarian 1	50
2	Technician 1	85
3	Herd Veterinarian 2	64
4	Herd Veterinarian 2	19
5	Technician 2	7
6	Technician 2	18
7	Technician 2	11
	Total number of samples:	**254**

Table 1 displays the seven different source of blood samples we utilized in this study along with the range of sample collectors who collected these samples. Over the course of the study, a total of 254 metabolic profiles were processed from doe serum samples. However, CentriVet handheld meter data was only collected or collected properly on 188 of the doe blood samples so our results will be based on the 188 samples which were tested with the meters and processed as metabolic profiles. Of these 188 samples, we censored 2 BHB values and 4 glucose values due to the values being not biologically sound. These censored values accounted for 1.06% of BHB values and 2.12% of glucose values.

Table 2. Population demographics for 188 blood samples from dairy goats in late pregnancy

Measurement	Glucose (Meter)	Glucose (Lab)	BHB (Meter)	BHB (Lab)
N	229	223	228	223
Mean	73.5	59.92	14.76	17.99
Standard Deviation	32.75	30.92	18.98	33.06
25th Percentile	59	45	3.12	3.23
75th Percentile	78	65	15.63	14.6

3.3.1 BHB

The BHB values measured by the CentriVet handheld meter, and the laboratory values had a high association ($r^2 = 0.877$, $P < 0.001$). Therefore, the CentriVet handheld meter was

found to be highly predictive of doe serum BHB concentration when using whole blood (Figure 1). The regression fit plot equation is y = 1.02(+/-0.03) x (meter value) – 1.41(+/-0.65), this equation can be used when predicting laboratory BHB serum concentrations given a meter value (Figure 1).

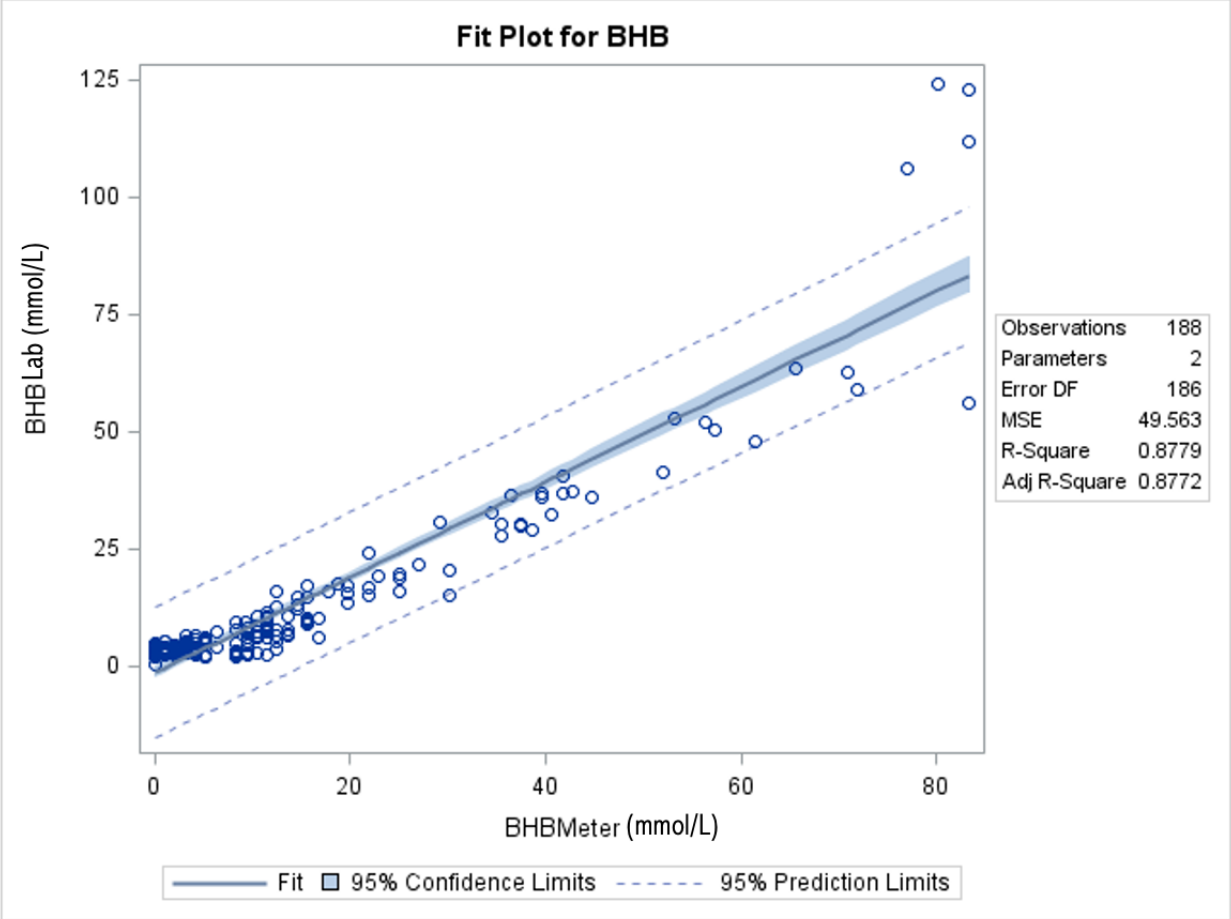

Figure 1. Fit plot illustrating 95% confidence and prediction limits for the whole blood BHB concentration from the CentriVet hand-held meter and the laboratory serum concentration

Residual values were calculated by subtracting the meter BHB concentrations from the laboratory serum BHB concentrations. The residual plot displayed a good association between hand-held meter and laboratory BHB values with the residuals well balanced over 0 (Figure 2).

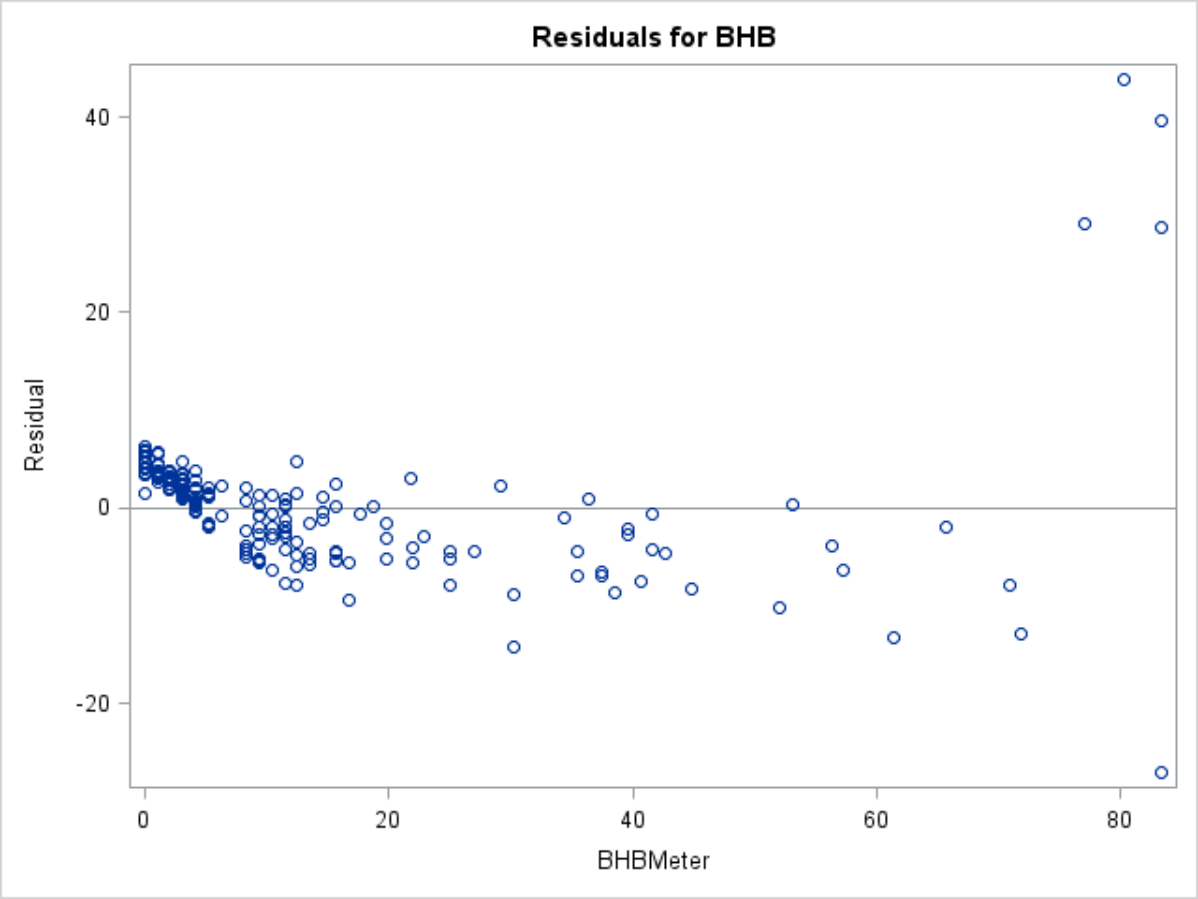

Figure 2. Residual plot for the whole blood BHB concentrations from the CentriVet hand-held meter versus the laboratory serum concentrations

3.3.2 Glucose

The glucose values measured by the CentriVet handheld meter, and the laboratory values had a high association ($r^2 = 0.852$, $P < 0.001$). Therefore, the CentriVet handheld meter was found to be highly predictive of doe serum glucose concentration when using whole blood (Figure 3). The regression fit plot equation is y = 0.835(+/-0.03) x (meter value) – 1.71(+/-2.07), this equation can be used when predicting laboratory measured glucose serum concentrations given a meter value (Figure 3).

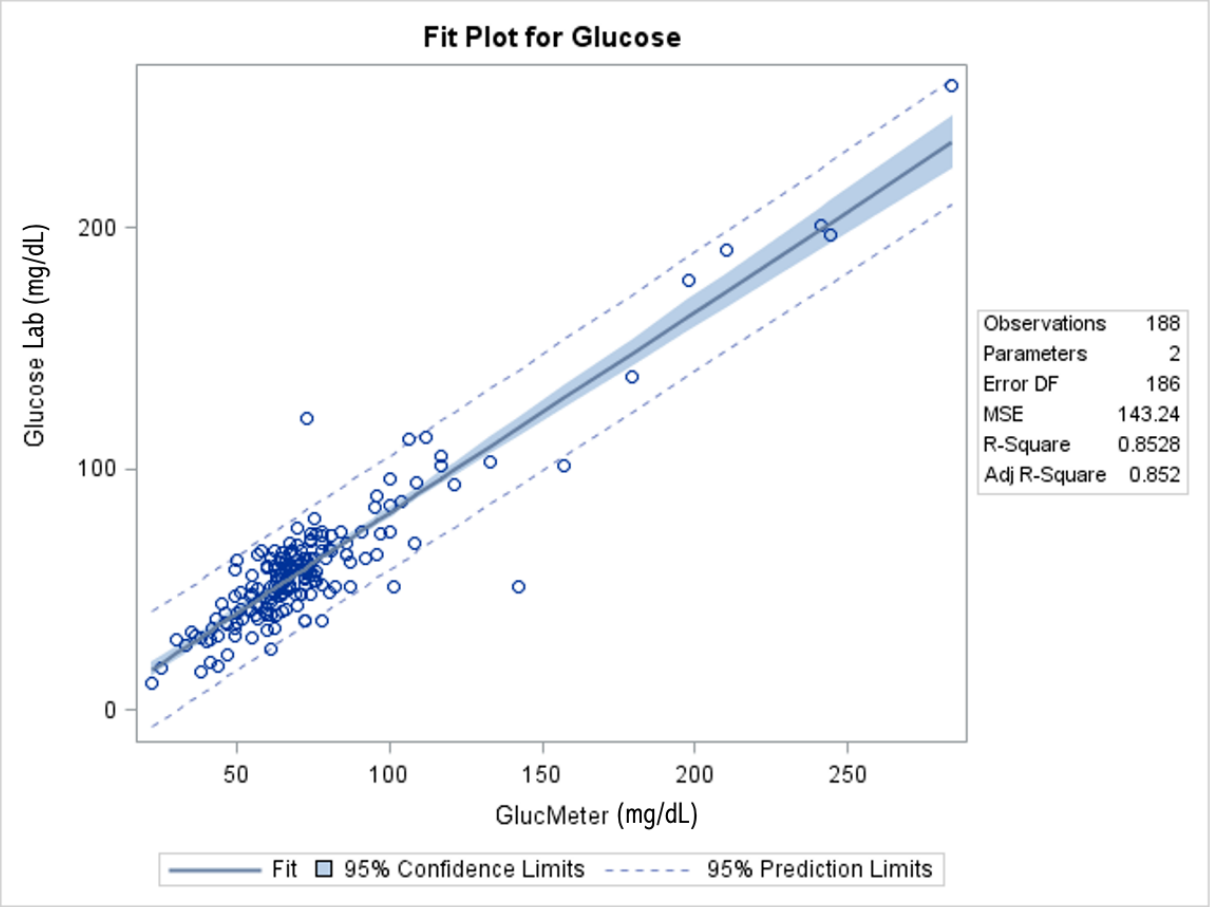

Figure 3. Fit plot illustrating 95% confidence and prediction limits for the whole blood glucose concentration from the CentriVet hand-held meter and the laboratory serum concentration

Residual values were calculated by subtracting the meter glucose concentrations from the laboratory serum glucose concentrations. The residual plot displayed a good association between hand-held meter and laboratory glucose values with the residuals well balanced over 0 (Figure 4).

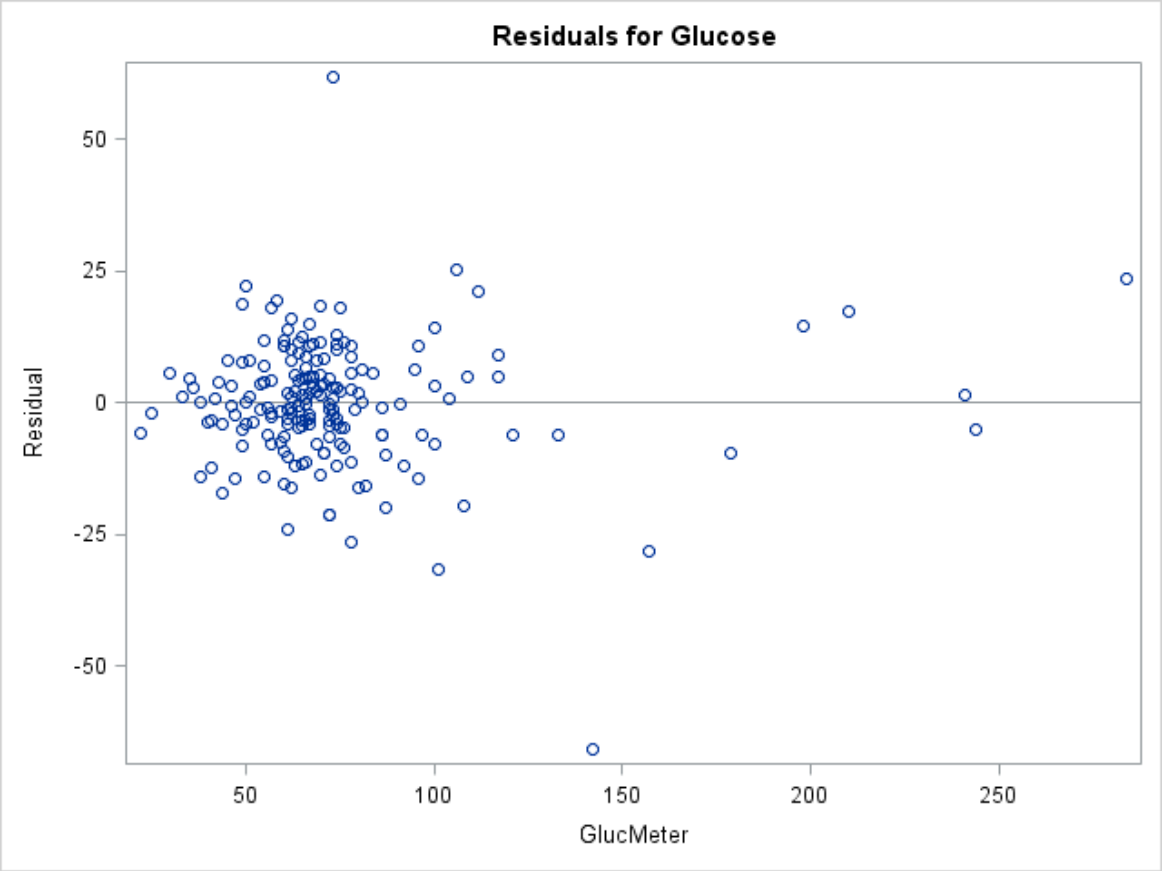

Figure 4. Residual plot for the whole blood glucose concentrations from the CentriVet hand-held meter versus the laboratory serum concentrations

For both glucose and BHB regression fit plots, the CentriVet hand-held meter was validated as highly predictive of the blood values especially at lower concentrations which is crucial for the diagnosis of subclinical and early pregnancy toxemia (Figure 1, 3).

3.4 Discussion

The results indicate that a strong correlation exists between the laboratory and meter measured BHB and glucose values. Therefore, from the results, the CentriVet Blood Glucose and Ketone Monitoring Meter was validated and based on the association, our findings suggest the

meter is an adequate tool to measure BHB and glucose. Compared to other studies focused on validating handheld meters, our BHB association was high but slightly lower in comparison to other studies with an r^2 of 0.877 (Panousis et al., 2012; Pineda and Cardoso, 2015; Doré et al., 2013). When compared to other studies, we had a large sample size of 188 samples which were measured with the meter and laboratory data. In Pichler's study, only 28 dairy goats were sampled from a single farm whereas in Doré's study 114 dairy goats were sampled (Doré et al., 2013, Pichler et al., 2014b). Our study had multiple different sample collectors, all of which who took the samples in the field versus a controlled environment, which may account for a lower association between meter and lab values in comparison to other studies. However, our glucose association ($r^2 = 0.852$) was higher in comparison to other studies (Panousis et al., 2012; Pichler et al., 2014b). The high association between glucose meter and lab values is an exciting finding which should be further explored to determine if the CentriVet Blood Glucose and Ketone Monitoring Meter is the best option for glucose measurements in dairy goats.

The CentriVet Blood Glucose and Ketone Monitoring Meter is calibrated for lower concentrations which reflect the threshold at which dairy goats may pass from normal to subclinical to clinical pregnancy toxemia. This calibration can allow for the meter to better differentiate affected versus unaffected dairy goats. Five BHB concentrations existed beyond the 95% confidence interval at the higher end of BHB concentrations. However, these are not clinically relevant because for disease diagnosis, it is more important that the meter can differentiate lower to slightly higher BHB values to indicate subclinical and clinical pregnancy toxemia in does (Figure 1).

3.5 Conclusion

The CentriVet Blood Glucose and Ketone Monitoring Meter has been validated as a tool which can accurately measure blood glucose and BHB values as an on-farm diagnostic tool. This tool has shown a strong association to laboratory testing measurements when used under field conditions. The meter may be better suited for small ruminants because of its test strips having a lower scale for ketogenic activity. Producers and veterinarians can utilize the CentriVet Blood Glucose and Ketone Monitoring Meter to measure doe blood glucose and BHB as a cost effective and quick diagnostic tool for pregnancy toxemia.

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Chapter 4

Metabolic Indicators of Pregnancy Toxemia and Prognosis

4.1 Introduction

Pregnancy toxemia results during late gestation for pregnant does, frequently with multiple fetuses, due to the large glucose requirement of fetuses and the doe's inability to adapt to the metabolic challenge (Marteniuk and Herdt, 1988). When a doe is undergoing a negative energy balance, resulting from insufficient glucose, the production of NEFAs and ketone bodies, such as BHB, for energetic purposes increases to reduce glucose use to help maintain blood glucose concentration (Herdt, 2000). Therefore, during pregnancy toxemia, BHB, glucose, and NEFA values are affected because of the doe's gluconeogenic response and metabolic disease (Herdt, 2000).

Limited research exists which explores blood parameter changes beyond BHB, glucose, and NEFAs associated with pregnancy toxemia in dairy goats. Furthermore, limited research exists which explores the relationship between BHB, glucose, and NEFA with various other blood chemistry parameter changes which occur during pregnancy toxemia. Vasava et al. (2016) did research comparing blood parameters between 20 healthy does and 45 does with pregnancy toxemia. In the study, Vasava et al. (2016) found that in does with pregnancy toxemia, there was significantly decreased glucose and calcium values and significantly increased SGPT, SGOT, BUN, creatinine, BHBA, and NEFA values. In a study focused on ewes with pregnancy toxemia, Khames Mustafa et al. (2023) found ewes with pregnancy toxemia had significant decreases in glucose, cholesterol, total protein, albumin, and globulin when compared to healthy ewes. Khames Mustafa et al. (2023) also found significant increases in BHB, NEFAs, triglycerides,

total bilirubin, ALT, ASP, ALP, and GGT in ewes with pregnancy toxemia when compared to healthy ewes.

Prevention and a timely diagnosis are extremely important to ensure adequate time for medical intervention for does with pregnancy toxemia. By exploring other blood chemistry parameter changes, a better understanding of pregnancy toxemia and prognosis in affected does may be achieved. Additionally, with a better understanding of the disease, new diagnostic or treatment methods may arise. We hypothesize that a parameter other than glucose or BHB can provide information on pregnancy toxemia. Exploring metabolic blood parameters and their relationships with BHB, glucose, and NEFAs in does with pregnancy toxemia will be the focus of this research study. This paper will discuss the materials and methods, results, discussion, and conclusions of the research project.

4.2 Materials and Methods

All procedures were reviewed and approved by the Pennsylvania State University Institutional Animal Care and Use Committee (IACUC#: PRAMS201747925).

4.2.1 Sample Collection

Whole blood was collected into blood tubes containing no anticoagulants or additives. Whole blood was collected from late pregnant does among 7 farms over a period between 2018 and 2023. Sampled does were any animal in the last month of pregnancy irrespective of presentation of clinical signs associated with pregnancy toxemia. Farm veterinarians or

veterinary technicians collected whole blood samples for immediate testing and harvesting of serum. All samples collected were organized within a spreadsheet along with the date collected and with any relevant doe information including doe birth date, date kidded and sampled, doe health history, doe survival, kid number born, gender, and their mortality or survivability.

4.2.2 Sample Analysis

The blood sample was either processed on the farm to harvest serum to send to our laboratory or directly returned to our laboratory for processing. Participating farms not local to Penn State University were provided a centrifuge for serum harvesting as needed. All harvested serum samples were catalogued and frozen (-80°C) until sent for analysis. Catalogued serum samples were batched and sent to the Oregon State Veterinary Diagnostic Laboratory, a commercial laboratory, to perform their metabolic profile clinical chemistry analysis. This lab's metabolic profile included clinical chemistry measures of blood urea nitrogen (BUN), creatinine, glucose, cholesterol, triglycerides, total protein, albumin, total bilirubin, creatine kinase (CK), gamma-glutamyl transferase (GGT), aspartate aminotransferase (AST), sodium, potassium, chloride, calcium, phosphorus, magnesium, total carbon dioxide, sorbitol dehydrogenase (SDH), BHB, and non-esterified fatty acids. All clinical chemistry parameters were determined using an AU480 chemistry analyzer according to manufacturer methods (Beckman Coulter Life Sciences, Indianapolis, IN). Measured bicarbonate concentration using an enzymatic method was reported as total carbon dioxide. Anion Gap was calculated using the following calculation $(Na^+ + K^+) - (CL^- + HCO_3^-)$ with all values in mEq/L.

4.2.3 Statistical Analysis

All measures were evaluated for normality using Proc Univariate (SAS ver. 9.4, Cary, NC). Measured parameters found not to be normally distributed were transformed using either natural logarithm or reciprocal functions. Population demographics were determined by the Proc Means procedure. Spearman correlation coefficients were determined for all measured parameters. Analysis of variance (ANOVA) using general linear model method (Proc GLM) provided statistical assessment of relationships between selected clinical chemistry parameters. Models to assess glucose and BHB method alignment included main effect of meter and farm as a covariate. Parameters BHB, NEFA, and glucose were categorized based on thresholds associated with health, subclinical, and clinical disease were related to other clinical chemistry parameters using ANOVA methods. The model statement included main effects of BHB, NEFA, or Glucose category (0,1,2), farm, and their interaction. Statistical significance was accepted at $P \leq 0.05$ with a tendency defined as $P > 0.05$ and ≤ 0.10.

4.3 Results

254 metabolic profiles were collected with 225 metabolic profiles being fully complete and used in our statistical analysis and results. The Proc Means Procedure analyzed the data and determined the population demographics which are displayed in table 3.

Table 3. Metabolic profile data for 225 late pregnant does

Parameter	N	Min	Max	Mean	Median	25th Percentile	75th Percentile	Std Dev	Std Error
GlucMeter	229	22	284	73.50	68	59	78	32.75	2.16
BHBMeter	228	0.00	83.33	14.76	8.33	3.12	15.63	18.98	1.26
BUN	225	5.00	174	20.49	18	15	22	16.14	1.08
Creatinine	225	0.10	3.70	0.61	0.60	0.50	0.70	0.34	0.02
Glucose	223	11	259	59.92	56	45	65	30.92	2.07
Cholesterol	223	37	221	83.04	80	64	96	26.97	1.81
Triglycerides	225	6.0	86	20.92	19	13	25	10.8	0.72
TotProt	225	3.70	8.60	6.39	6.30	5.80	6.90	0.85	0.06
Albumin	225	1.70	4.30	3.02	3.00	2.80	3.30	0.44	0.03
TotBilirubin	225	0.10	2.10	0.26	0.20	0.10	0.30	0.27	0.02
CK	224	32	1738	154.6	100	70.5	175.5	165.48	11.06
GGT	224	1.00	103	31.23	30.5	23	38	13.7	0.92
AST	225	37	612	93.72	76	61	97	68.2	4.55
Sodium	225	97	176	142.96	145	143	148	10.17	0.68
Potassium	225	2.70	11.1	4.83	4.50	4.20	5.00	1.20	0.08
Chlorine	225	71	134	105.93	108	104	110	7.95	0.53
Calcium	225	3.60	11.1	8.19	8.20	7.50	9.00	1.17	0.08
Phosphorus	223	2.20	17.20	6.34	5.90	5.00	7.40	2.14	0.14
Magnesium	225	1.50	4.70	2.59	2.60	2.30	2.80	0.46	0.03
tCO_2	225	3.20	30.30	21.32	22.1	20.00	24.2	4.84	0.32
SDH	202	0.10	282	29.42	20.8	15.1	29.6	35.99	2.53
BHB	223	0.11	181.2	17.99	5.34	3.23	14.6	33.06	2.21
NEFA	224	0.05	2.22	0.65	0.42	0.21	1.03	0.55	0.04
Anion Gap	225	12	37.1	20.52	19.1	17	23	4.84	0.32

Although data was gathered on some dairy goats regarding the health condition of the does and the survival of the doe and kids, the data was incomplete and was not used for a comparison of animal health status to the metabolic profile data. Instead, we took three key parameters, BHB, glucose, and NEFAs, and made categories based on published numbers to analyze for pregnancy toxemia.

To analyze the metabolic profile characteristics in relation to BHB, NEFA, and glucose, categories were formulated. Categories were based on historical data on pregnancy toxemia and the deviations from normal which occur during clinical disease. Metabolic profiles falling under BHB category 0 had a BHB value less than 8.6 mg/dL. Metabolic profiles falling under BHB category 1 had a BHB value greater than 8.59 mg/dL but less than 25 mg/dL. Metabolic profiles falling under BHB category 2 had a BHB value greater than 24.9 mg/dL. Metabolic profiles falling under glucose category 0 had a glucose value less than 40 mg/dL. Metabolic profiles falling under glucose category 1 had a glucose value greater than 39.9 mg/dL but less than 90 mg/dL. Metabolic profiles falling under glucose category 2 had a glucose value greater than 89.9 mg/dL. Metabolic profiles falling under NEFA category 0 had a NEFA value less than 0.4 mg/dL. Metabolic profiles falling under NEFA category 1 had a NEFA value greater than 0.39 mg/dL but less than 0.8 mg/dL. Metabolic profiles falling under NEFA category 2 had a NEFA value greater than 0.79 mg/dL.

By examining the relationships between BHB, Glucose, and NEFA values with the blood chemistry parameters we were able to identify the statistically significant relationships between the categories. These relationships and their significances are displayed in tables 4-6.

Table 4. BHB Categories relationship with blood parameters (mg/dL)

Parameter	x < 8.6 (n = 151)	8.6 < x < 25 (n = 36)	x > 24.9 (n = 38)	Pr < F
BUN	20.21 ± 18.74	20.53 ± 10.57	21.61 ± 6.85	NS
Creatinine	0.54 ± 0.26^{a}	0.69 ± 0.28^{ab}	0.82 ± 0.54^{b}	0.0115
Glucose	64.74 ± 29.07^{a}	55.57 ± 39.17^{a}	44.35 ± 23.67^{b}	0.0009
Cholesterol	84.36 ± 23.08^{a}	87.39 ± 41.30^{b}	73.76 ± 22.52^{b}	0.0007
Triglycerides	21.01 ± 10.27^{b}	18.42 ± 10.49^{b}	22.89 ± 12.82^{a}	0.0536
TotProt	6.48 ± 0.90	6.26 ± 0.68	6.16 ± 0.75	NS
Albumin	3.07 ± 0.45^{a}	3.03 ± 0.42^{a}	2.79 ± 0.35^{b}	0.0350
TotBilirubin	0.19 ± 0.21	0.49 ± 0.40	0.33 ± 0.17	NS
CK	119.70 ± 101.23^{b}	192.08 ± 175.12^{ab}	256.87 ± 278.78^{a}	0.0025
GGT	32.81 ± 13.10	27.50 ± 17.05	28.55 ± 11.57	NS
AST	88.41 ± 71.46	104.44 ± 77.10	104.63 ± 38.57	NS
Sodium	142.13 ± 10.15	147.14 ± 3.40	142.34 ± 13.31	NS
Potassium	4.71 ± 0.93^{b}	5.34 ± 1.69^{a}	4.82 ± 1.48^{a}	< 0.0001
Chlorine	105.84 ± 8.08^{b}	108.64 ± 3.67^{a}	103.74 ± 9.64^{b}	0.0360
Calcium	8.58 ± 1.04^{a}	7.65 ± 0.79^{a}	7.17 ± 1.16^{b}	0.0045
Phosphorus	5.83 ± 1.97^{b}	7.02 ± 1.75^{b}	7.70 ± 2.39^{a}	0.0532
Magnesium	2.60 ± 0.41	2.66 ± 0.47	2.52 ± 0.61	NS
tCO_2	22.63 ± 3.15^{a}	21.97 ± 2.05^{a}	15.48 ± 7.41^{b}	< 0.0001
SDH	26.49 ± 32.45^{b}	21.68 ± 16.50^{b}	50.56 ± 54.64^{a}	0.0017
BHB	4.26 ± 1.79^{c}	14.20 ± 4.01^{b}	75.41 ± 48.70^{a}	< 0.0001
NEFA	0.36 ± 0.30^{c}	1.15 ± 0.45^{b}	1.33 ± 0.46^{a}	<0.0001
Anion Gap	18.32 ± 3.11^{c}	21.88 ± 2.70^{b}	27.94 ± 4.20^{a}	< 0.0001

NS= Not Significant ($P > 0.05$)

For BHB categories, several blood chemistry parameters displayed a significant relationship with BHB fluctuation as seen in Table 4. These parameters with significant relationships with BHB included creatinine, glucose, cholesterol, triglycerides, albumin, CK, potassium, chlorine, calcium, phosphorus, total CO_2, SDH, NEFA, and Anion Gap ($P \leq 0.05$). The blood parameters with highly significant relationships ($P < 0.0001$) with BHB categories were potassium, tCO_2, NEFA, and anion gap.

Table 5. Glucose Categories relationship with blood parameters (mg/dL)

Parameter	x < 40 (n = 41)	39.9 < x < 90 (n = 166)	x > 89.9 (n = 18)	Pr < F
BUN	18.51 ± 4.85^{b}	18.98 ± 6.48^{b}	39.00 ± 50.76^{a}	< 0.0001
Creatinine	0.69 ± 0.21^{b}	0.55 ± 0.20^{b}	1.05 ± 0.90^{a}	< 0.0001
Glucose	31.08 ± 7.54^{c}	57.95 ± 10.12^{b}	140.61 ± 50.20^{a}	<0.0001
Cholesterol	81.95 ± 34.53	84.20 ± 25.63	75.06 ± 17.62	NS
Triglycerides	20.37 ± 13.14	21.54 ± 10.43	16.39 ± 7.01	NS
TotProt	6.07 ± 1.00^{b}	6.46 ± 0.81^{ab}	6.52 ± 0.72^{a}	0.0161
Albumin	2.88 ± 0.55	3.06 ± 0.40	2.95 ± 0.47	NS
TotBilirubin	0.45 ± 0.41	0.21 ± 0.21	0.26 ± 0.16	NS
CK	212.88 ± 174.50^{ab}	124.62 ± 97.44^{b}	296.72 ± 397.05^{a}	0.0020
GGT	22.93 ± 12.14^{b}	32.34 ± 12.59^{b}	40.00 ± 18.04^{a}	0.0047
AST	100.12 ± 35.49	87.67 ± 67.99	134.83 ± 105.90	NS
Sodium	138.95 ± 14.60^{b}	144.07 ± 8.77^{a}	141.89 ± 7.90^{a}	0.0002
Potassium	5.63 ± 2.02	4.64 ± 0.78	4.75 ± 1.24	NS
Chlorine	102.59 ± 10.25^{b}	106.96 ± 7.13^{a}	104.11 ± 7.14^{a}	0.0030
Calcium	7.11 ± 1.21^{b}	8.51 ± 1.00^{a}	7.72 ± 0.89^{a}	0.0001
Phosphorus	7.32 ± 2.04^{ab}	5.99 ± 1.57^{b}	7.29 ± 4.69^{a}	0.0414

Magnesium	2.44 ± 0.54[c]	2.61 ± 0.39[b]	2.81 ± 0.71[a]	< 0.0001
tCO_2	17.45 ± 6.65[b]	22.36 ± 3.59[a]	20.44 ± 5.62[a]	< 0.0001
SDH	43.44 ± 53.32[a]	26.61 ± 32.71[b]	30.38 ± 19.29[b]	0.0465
BHB	47.41 ± 45.24[a]	11.24 ± 25.18[b]	14.50 ± 31.18[b]	< 0.0001
NEFA	1.16 ± 0.52	0.52 ± 0.49	0.72 ± 0.44	NS
Anion Gap	24.53 ± 4.61[a]	19.35 ± 4.21[b]	22.13 ± 5.67[ab]	0.0103

NS= Not Significant ($P > 0.05$)

For glucose categories, several blood chemistry parameters displayed a significant relationship with glucose fluctuation as seen in Table 5. These parameters with significant relationships with BHB included BUN, creatinine, total protein, CK, GGT, sodium, chlorine, calcium, phosphorus, magnesium, total CO2, SDH, and BHB ($P \leq 0.05$). The blood parameters with highly significant relationships ($P < 0.0001$) with glucose categories were BUN, creatinine, magnesium, tCO_2, and BHB.

Table 6. NEFA Categories relationship with blood parameters (mg/dL)

Parameter	**x < 0.4 (n = 106)**	**0.39 < x < 0.8 (n = 49)**	**x > 0.79 (n = 70)**	**Pr < F**
BUN	21.56 ± 22.06[a]	19.10 ± 9.42[b]	19.86 ± 6.35[b]	0.0215
Creatinine	0.52 ± 0.29	0.72 ± 0.52	0.68 ± 0.19	NS
Glucose	62.75 ± 23.46	64.22 ± 42.61	52.40 ± 30.33	NS
Cholesterol	83.09 ± 19.57[a]	84.47 ± 32.55[a]	81.99 ± 32.08[b]	0.0156
Triglycerides	22.81 ± 11.00	19.86 ± 13.08	18.79 ± 8.04	NS
TotProt	6.53 ± 0.90	6.26 ± 0.92	6.28 ± 0.69	NS
Albumin	3.07 ± 0.44	2.95 ± 0.51	3.00 ± 0.39	NS
TotBilirubin	0.14 ± 0.15[b]	0.26 ± 0.29[ab]	0.44 ± 0.30[a]	0.0328
CK	109.58 ± 78.75	181.51 ± 262.55	203.30 ± 158.73	NS

GGT	33.99 ± 12.16	31.86 ± 15.13	26.66 ± 13.84	NS
AST	79.58 ± 56.49	109.86 ± 89.49	103.81 ± 63.87	NS
Sodium	142.04 ± 10.06	141.86 ± 11.60	145.14 ± 8.99	NS
Potassium	4.62 ± 0.68	4.66 ± 1.30	5.25 ± 1.60	NS
Chlorine	105.89 ± 8.15	105.57 ± 9.19	106.26 ± 6.72	NS
Calcium	8.76 ± 1.03^{a}	8.06 ± 1.12^{a}	7.41 ± 0.90^{b}	0.0136
Phosphorus	5.88 ± 2.07	6.15 ± 2.32	7.15 ± 1.90	NS
Magnesium	2.60 ± 0.39	2.65 ± 0.60	2.55 ± 0.45	NS
tCO_2	22.84 ± 3.19^{a}	20.69 ± 6.00^{ab}	19.45 ± 5.29^{b}	0.0139
SDH	24.19 ± 26.52	42.59 ± 59.00	28.54 ± 23.09	NS
BHB	3.75 ± 1.57^{c}	21.98 ± 43.12^{b}	36.61 ± 39.69^{a}	0.0002
NEFA	0.22 ± 0.09^{c}	0.57 ± 0.11^{b}	1.37 ± 0.37^{a}	<0.0001
Anion Gap	17.88 ± 2.88^{c}	20.25 ± 4.92^{b}	24.69 ± 4.26^{a}	<0.0001

NS= Not Significant ($P > 0.05$)

For NEFA categories, several blood chemistry parameters displayed a significant relationship with NEFA fluctuation as seen in Table 6. These parameters with significant relationships with NEFA included BUN, cholesterol, total bilirubin, phosphorus, tCO2, BHB, and anion gap ($P \leq 0.05$). The blood parameter with a highly significant relationship ($P < 0.0001$) with NEFA categories was the anion gap.

Table 7. Summary of significant relationships between BHB, glucose, and NEFA categories and blood parameters

Parameter	BHB Categories	Glucose Categories	NEFA Categories
BUN		x	x
Creatinine	x	x	
Glucose	x	x	
Cholesterol	x		x

Triglycerides	x		
TotProt		x	
Albumin	x		
TotBilirubin			x
CK	x	x	
GGT		x	
AST			
Sodium		x	
Potassium	x		
Chlorine	x	x	
Calcium	x	x	x
Phosphorus		x	
Magnesium		x	
tCO_2	x	x	x
SDH	x	x	
BHB	x	x	x
NEFA	x		x
Anion Gap	x	x	x

An x signifies a significant ($P \leq 0.05$) relationship between the categories

4.4 Discussion

The metabolic profiles displayed different metabolic status criteria based on BHB levels which would suggest there are other blood parameters which can be examined when evaluating a doe for pregnancy toxemia. There were several significant ($P \leq 0.05$) relationships between the three BHB categories and the changing blood parameters as displayed in Table 7. These

relationships suggest that when BHB reaches a certain threshold, increasing from 8.6 mg/dL to a value between 8.6 and 25 mg/dL to values greater than 24.9 mg/dL, there are significant changes to other blood parameter values. Notably, when analyzing BHB categories and NEFA categories there were highly significant relationships between NEFA and BHB changes. Each BHB category generated a significant NEFA value, meaning there was a significant difference in NEFA value between the three thresholds for BHB ($P < 0.0001$). Likewise, each NEFA category generated a significant BHB value, meaning there was a significant difference in BHB value between the three thresholds for NEFA ($P = 0.0002$). As NEFA increased, there were significant increases in BHB and as BHB increased there were significant increases in NEFA at each threshold level. This relationship further supports the idea that NEFA mobilization of body fat is strongly related to ketone body synthesis. When the pregnant doe is mobilizing their body fats during gluconeogenesis, there are increased levels of NEFAs within circulation, which stimulates the uptake of fatty acids from the liver and the production of ketone bodies (Schlumbohm and Harmeyer, 2004). An increased concentration of ketone bodies within circulation results in a decrease in the hepatic rate of glucose production, furthering the progression of pregnancy toxemia and the production of NEFA (Schlumbohm and Harmeyer, 2004). Therefore, BHB and NEFA can not only be determinants of a doe's pregnancy toxemia status and can also be used to analyze the doe's ketone body production when given a NEFA value and vice versa.

As discussed, a high BHB value has been and continues to be a proven indication of pregnancy toxemia in does. A notable finding when examining the BHB categories and the changing blood parameters was the highly significant relationship between BHB and the anion gap as well as the relationship between BHB and total CO_2 ($P < 0.0001$). The relationship demonstrated that as BHB increased anion gap increased significantly while tCO_2 decreased

significantly. This relationship with BHB and anion gap and tCO_2 is related to acid base balance and the sensitization of the doe to metabolic acidosis due to the large ketone body production. When ketone bodies are generated, the acid base balance is disturbed within the doe due to the high acidity of ketone bodies.

This disruption of the acid base balance, as demonstrated with anion gap and tCO_2, also plays a role in pregnancy toxemia's relationship with glucose. When the doe experiences a large increase in ketone bodies, their body tries to get rid of them by either urinating them out or the doe will stop eating. If the doe stops eating due to the large amount of ketone bodies in circulation, the doe is put into a glucose challenge. The data demonstrates how glucose homeostasis is maintained even when fat is mobilized and when NEFA increases. Within NEFA categories and within glucose categories, as NEFA and glucose increased there was not a significant relationship between the two parameters ($P > 0.05$). This demonstrates how total loss of glucose homeostasis is not an essential component to the pregnancy toxemia disease process. BHB and glucose did display a significant relationship with fluctuations between the BHB and glucose categories. For BHB categories, glucose significantly decreased when the BHB value was greater than 24.9 mg/dL ($P = 0.0009$). For glucose categories, BHB significantly decreased when the glucose value was greater than 39.9 mg/dL ($P < 0.0001$). This relationship supports the historical data and notes the relationship of increased ketone body production when glucose is limited. When glucose is high, there is less of a need for ketone bodies for energy production which may be the main reason BHB declines when glucose reaches the 39.9 mg/dL or higher threshold.

4.5 Conclusion

There were many significant ($P \leq 0.05$) relationships between BHB, NEFA, glucose and different metabolic parameters. These metabolic profiles can be used to look at different statuses of the pregnant animal to determine their health state in relation to pregnancy toxemia. The finding that glucose homeostasis disruption may not be a requirement for pregnancy toxemia suggests how BHB and NEFA may be better indicators of pregnancy toxemia status in a pregnant doe. The relationship between BHB and anion gap and tCO_2 reflects an acid base disruption relationship. This relationship between acid base disruption and pregnancy toxemia can be further researched to better understand and develop treatment plans for does with pregnancy toxemia. A reduction in acid base status suggests treatment of animals may need to involve fluids therapy with base deficit. Some of the other significant relationships between the three categories should be further researched to further understand the disease process.

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Chapter 5

Conclusions

The results of the study have supported the validation of the CentriVet Handheld Blood Glucose and Ketone Monitoring Meter for use in measuring BHB and glucose in goats at the farm level. The study design was such that multiple operators utilized the hand-held meter to measure the BHB and glucose content rather than a single operator in a laboratory. Therefore, the CentriVet Handheld Blood Glucose and Ketone Monitoring Meter can be used in the field to help with an earlier and cost-effective diagnosis of pregnancy toxemia.

The analysis of metabolic profiles in does using BHB, NEFA, and glucose parameters demonstrated different significant relationships which can occur in does with pregnancy toxemia. NEFA and BHB displayed a highly significant relationship supporting the idea that NEFA mobilization of body fat is strongly related to ketone body synthesis and that these processes occur during pregnancy toxemia as a contributor from a negative energy balance.

Further metabolic analysis using blood chemistry profiles suggests glucose alone is not as diagnostic for potential outcome due to glucose homeostasis disruption not being required for the disease process to advance. Acid base balance, looking at anion gap and tCO_2, displayed a significant disruption with increasing BHB values and may be a key process to examine to make better treatment plans in the future.

Further changes should be researched by looking at the significant relationships based on category parameters. These changes could be studied and used to impact treatment, prognosis, and the overall understanding of the pregnancy toxemia disease process.

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