THE PENNSYLVANIA STATE UNIVERSITY
SCHREYER HONORS COLLEGE

DEPARTMENT OF VETERINARY AND BIOMEDICAL SCIENCES

EVALUATING GOAT COLOSTRUM QUALITY AND KID PASSIVE TRANSFER STATUS

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SPRING 2020

A thesis
submitted in partial fulfillment
of the requirements
for a baccalaureate degree
in Veterinary and Biomedical Sciences
with honors in Veterinary and Biomedical Sciences

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ABSTRACT

Feeding high-quality colostrum is the most important event in the newborn kid's life to ensure viability and survivability. The study objectives were to evaluate goat colostrum quality by defining a relationship between Brix and immunoglobulin G (IgG) concentration, document serum IgG concentration based on total IgG consumed, and determine a relationship between total protein and serum IgG concentrations. All samples were collected on a 900-doe commercial dairy goat facility. Goat colostrum was evaluated for IgG concentration using a Brix refractometer and radial immunodiffusion (RID). Kid blood samples were evaluated for serum total protein with a digital refractometer and serum IgG concentration with goat IgG enzyme-linked immunosorbent assay (ELISA). Mean ± SD (median, range) colostrum IgG for all samples (n=114) was 71.0 ± 36.8 mg/mL (74.2, 4.2-180.5). Brix and RID measures were highly correlated ($P<0.0001$, $r_2=0.85$). Brix determinations tended to be influenced by dry length ($P=0.084$), but not age or number of kids. Linear regression model relating Brix to RID in goat colostrum was RID (mg/mL)=6.97(Brix)-73.65 ($r_2=0.73$, $P<0.0001$). Mean ± SD (median, range) serum total protein (n=30) and total IgG concentration (n=57) were 6.0 ± 0.8 g/dL (6.0, 4.5-7.2) and 15.8 ± 7.3 mg/mL (15.3, 3.1-36.1), respectively. Serum total protein measured was highly associated ($r_2=0.73$; $P<0.0001$) with serum IgG concentration. Total IgG consumed showed the greatest influence ($P=0.0020$) with no effect of method of feeding or time to feeding. Calculated efficiency of IgG absorption was 16.8 ± 8.8% (16.4; 3.6-52.9). Based on these results, the digital Brix refractometer is a valid method to predict colostrum quality based on IgG concentration in goats. Serum total protein is an effective method to evaluate IgG in serum.

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ACKNOWLEDGEMENTS

I would like to thank everyone at the Central Milk Testing Lab (CMTL) who has helped me with this project. First, Rebekah Russell who selflessly helped me at 6 A.M. during the work week, on weekends during the summer, and mentored me throughout the lab process. I could not have conducted this research without you. Thank you to Louise Byler, who organized the statistics and walked me through the whole process. Thank you to Jen, who graciously shared her lab space with me and taught me how to tackle the enzyme-linked immunosorbent assay (ELISA).

I would also like express my gratitude to the Penn State University Veterinary and Biomedical Sciences (VBSC) faculty including Dr. Acar, Dr. Griel, Dr. Brown, and Dr. Dodds who have played an integral role in my educational experience.

Lastly, I wish to thank Dr. Van Saun who has been my professor, advisor, and mentor. I have learned so much these last four years and appreciate your guidance and support through everything. Thank you for allowing me to conduct research with you.

Chapter 1

INTRODUCTION

Feeding of high-quality colostrum is the single most important event in life of a newborn ruminant to ensure viability and survivability. Colostrum provides agammaglobulinemic newborns with immunity though the passive transfer of immunoglobulins shortly after birth. Colostrum quality is based on the concentration of immunoglobulin G (IgG). The failure of passive transfer is the primary cause of increased susceptibility to infectious disease, increased morbidity, and increased mortality rates in newborn kids (O'Brien and Sherman, 1993). Therefore, high-quality colostrum is essential for kid health. In dairy cattle, there is extensive research on colostrum quality assessment with Brix (Bielmann et al., 2010) and passive transfer analysis (Beam et al., 2009), but this information is not available for goat kids.

The overall objective of this study was to evaluate colostrum quality in goats, determine if there exists a relationship between Brix reading and IgG concentration, document serum IgG concentrations in goat kids based on total IgG consumed, and determine the relationship between total protein and serum IgG concentration. A practical application of using a Brix refractometer for testing goat colostrum would be useful if a valid method.

To further understand goat colostrum quality, colostrum samples were collected and evaluated for IgG via Brix and the gold standard method, radial immunodiffusion (RID). Additional data relative to kidding was collected such as kid numbers, live vs. dead, birth weights, time from kidding, and lactation number to determine if these variables effected colostrum quality. To further understand passive transfer in goats, serum samples from kids were

collected and evaluated for total protein via a refractometer and IgG concentration via enzyme-linked immunosorbent assay (ELISA). The IgG of colostrum consumed was compared to the concentration of colostrum and total protein in kid serum. The null hypothesis tested was that Brix measure is not associated with goat colostrum IgG concentration and total protein is not associated with kid serum IgG concentration.

Chapter 2

LITERATURE REVIEW

2.1. Introduction

Feeding colostrum is vital within the first hours after birth since immunoglobulins are not transferred through the placenta during pregnancy in small ruminants, and there is a rapid reduction in intestinal absorption efficiency of immunoglobulins in the neonate. Kid goats need colostrum in the first hours of life as a source of immunoglobulin G (IgG), which provides immunity during the first month of life (Vihan, 1988). Studying this is important to ensure neonate vitality and future production implications such as improved average daily gain (Van Amburgh, 2018). The purpose of this literature review is to synthesize what is known about colostrum composition, colostrum evaluation methods, colostrum management strategies, passive transfer, and passive transfer evaluation. Colostrum quality and passive transfer is well researched in cattle; however, data is not comprehensive in goats.

2.2. Colostrum Composition

Colostrum is a unique mammary secretion in all mammals defined as the first milk produced just prior to and directly following parturition. Colostrum's critical role is to provide passive immunoglobulin transfer to newborns and to provide some level of immunologic protection until the adaptive immune response initiates. At birth, ruminants are agammaglobulinemic due to the inability of uterine and fetal tissues to transfer maternal immunoglobulin molecules (Halliday and Williams, 1979). Most farm animal species, including goat kids, rely on intestinal absorption of colostral immunoglobulins to provide passive humoral

immunity, and stimulation of other aspects of immune responses initiated by maternal lymphocytes and other immune regulators (Van Amburgh, 2018).

Colostrum provides essential nutrients and bioactive compounds that provide a variety of functions such as immunoglobulins and anti-infection agents to neonates (Pond et al., 2011). Specially, colostrum differs from mature milk due to its characteristic high concentration of IgG (Micusan and Borduas, 1977). Colostrum also contains neutrophils, lymphocytes, and various growth factors that are thought to play a role in neonate immune development (Pond et al., 2011). Growth factors in colostrum develop the gastrointestinal tract and enhance nutrient uptake and utilization (Hammon and Blum, 2012). Leukocytes in colostrum carry "maternal memory" from prior exposure to pathogenic organisms in the dam, which can improve the cellular immunity in the neonate (Van Amburgh, 2018). Beyond immune cells, colostrum contains other components of nutritional importance such as fat, proteins, and lactose that provide energy to support thermoregulation (Ontsouka et al., 2003).

Colostrum also contains vitamins and minerals that have numerous functions in the neonate. Goat colostrum contains niacin, pantothenic acid, riboflavin, thiamin, and vitamin A. Compared to cow milk, goat milk is deficient in pyridoxine, folate, and vitamin B_{12} (Jenness, 1980). Goat colostrum is a rich source of many minerals including sodium, calcium, magnesium, and phosphorus, which are important for maintaining osmotic balance in newborns (Rashid et al., 2012).

Colostrum composition such as protein, fats, IgG concentrations, etc. varies both across species and within breeds of goats. Table 1 displays the differences between goat and cow colostrum. Goat colostrum has higher average fat, but lower average total IgG levels compared to cow colostrum.

Table 1. Comparison of physiochemical characteristics of goat and cow colostrum

Characteristic	Goat[1]	Cow[2,3]
pH	6.38	6.32
Density (g/mL)	1.053	1.048
Fat (%)	9.5	6.7
Protein (%)	13.6	14.9
IgG (mg/mL)	28.2	35
Lactose (%)	2.9	2.5

[1]Data from Romero et al., 2013
[2,3] Data from Kehoe et al., 2008 (fat, protein, IgG, lactose) and McGrath et al., 2016 (pH, density).

2.3. Immunoglobulins

Immunoglobulins are a family of proteins that are the key immunological agent of newborn animals that are passed through colostrum. There are numerous types of immunoglobulins including IgG, immunoglobulin A (IgA), and immunoglobulin M (IgM). The relative proportion of immunoglobulin classes is highly dependent on species. In ungulate species such as horses, sheep, pigs, and goats, IgG is the primary immunoglobulin and is key for protection against bacterial and viral infections (Pond et al., 2011). There are subclasses of IgG in colostrum; IgG_1 and IgG_2 are the most common. These subclasses have the same general molecular structure with subtle differences in amino acid composition. IgG normally has a molecular mass around 160 kilodaltons and is monomeric. It consists of two identical heavy chains and two light chains which are linked together with disulfide bonds to create the typical Y-shape (Hurley and Theil, 2011). Micusan and Borduas (1977) observed that the concentration of normal adult mixed breed goat serum total IgG was 9.97 ± 1.55 mg/mL, where IgG_1 was 10.92 ± 0.84 mg/mL and IgG_2 was 9.07 ± 0.78 mg/mL. In goat colostrum, IgG concentration was

about 2.4-2.8 times greater than in serum and the IgG_1 subclass made up 95-98% of the total immunoglobulins (Micusan and Borduas, 1977). To measure the immunoglobulin proportions in goat serum, IgG_1 and IgG_2 were isolated via chromatography and measured via automated immune-precipitin reactions (AIR). This is the only study that has quantified IgG_1 and IgG_2 in goat serum and little data is available regarding AIR as a method to measure IgG.

Other than IgG, IgA and IgM are major classes of immunoglobulins that are present in mammals, including goats, but in lower relative proportion. These other forms of immunoglobins are different than IgG due to variation in the number and location of the disulfide bonds in the heavy chains. IgA and IgM are polymeric, in which the monomeric immunoglobulins link together through covalent interactions. IgA is found in the dimeric form and IgM is found in the pentameric form. IgA is present in mucosal secretions and plays a role in preventing mucosal infection by agglutinating microbes. IgM is produced initially after an organism is exposed to an antigen for the first time and has a low specificity and potency (Hurley and Theil, 2011).

Immunoglobulin-A concentration was 0 mg/mL in dairy kid goat serum at birth (Rodriguez et. al., 2009); however, IgA concentration was 0.45 mg/mL in neonatal dairy calves (Johnson et. al., 2007). Immunoglobulin-M concentration was 1.49 mg/mL at birth in neonatal dairy goats (Rodriguez et. al., 2009), and were 0 mg/mL in neonatal dairy calves (Johnson et. al., 2007). These differences in IgA and IgM between kids and calves may be attributed to species differences. In both goats and cattle, the immunoglobulins were both measured via enzyme linked immunosorbent assay (ELISA). Another study, Rudovsky et al. (2008), measured the different subclasses of immunoglobins in dairy goat colostrum at kidding via ELISA and reported IgG was 49.1 mg/mL, IgM was 3.19 mg/mL, and IgA was 2.00 mg/mL.

2.4. Importance of Colostrum

Numerous studies have documented the importance of colostrum and successful passive transfer of immunoglobulins for health, growth, and survival of animals. Failure of passive transfer occurs when not enough antibodies are absorbed after birth, which can increase the risk of infectious diseases. O'Brien and Sherman (1993) reported failure of passive transfer in kids at birth (defined when the serum IgG concentration is less than 12 mg/mL) leads to increased morbidity and mortality from infectious disease in young goats. A French survey found that 92% of kid goats deprived colostrum died within 2 days of birth (Morand-Fehr et al., 1984). Nandakumar and Rajagopalaraja (1983) measured serum immunoglobulin of kids of undefined breeds 18 hours after ingestion of colostrum and found a mean of 73.59 mg/mL (measured with Zinc Sulphate Turbidity Test which measures all immunoglobulins not just IgG). In the following two months, 44% of those with serum immunoglobulin concentrations below the mean died, whereas 3.8% of kids with a serum level above the mean died. Deceased kid immunoglobulin concentration was significantly lower than surviving kids, and mortality rate was significantly higher in kids with immunoglobulin concentration below the mean (Nandakumar and Rajagopalaraja, 1983). Overall these observations suggest a higher immunoglobulin concentration may have protected kids from infection and death.

In calves, there are long-term benefits documented with adequate colostrum intake such as improved rate of gain and feed efficiency (Robison et al., 1988), reduced age at first calving, and improved milk production (DeNise et al.,1989). In goats, similar long-term benefits have been documented showing increased rate of gain in relation to elevated serum IgG concentrations 24 hours after parturition (Massimini et al., 2007). Pritchett et al. (1991) found that a significant proportion of dairy calves (Holstein) suffer from failure of passive transfer,

which causes high mortality rates and costs the industry hundreds of millions of dollars every year. Any health event resulting from failure of passive transfer that detracts from feed intake can affect the pre-weaning growth rate, reducing the opportunity for enhanced milk yield as an adult (Van Amburgh, 2018)

2.5. Colostrogenesis

Colostrogenesis is the transfer of immunoglobulins from the blood into mammary secretions in the mother (Baumrucker et al., 2010). In ruminants, this process begins several weeks before parturition and stops directly prior to parturition (Brandon et al., 1971). In goat plasma, IgG concentration decreases by 38% from the third month of gestation until partition at five months (Castro et al., 2006). This transfer of immunoglobulins from dam serum to milk occurs so that milk has immune-boosting components for immunodeficient young.

The immunoglobulins are transferred though mammary epithelial cells via active receptor-mediated transport systems (Hurley and Theil, 2011). There are specific receptors for IgG_1 on the basilateral surface of alveolar epithelial cells found during colostrum formation (Leary et al., 1982). These receptors, which transport IgG into colostrum, are identified as the neonatal receptor (FcRn). The receptor is a heterodimer composed of a membrane-bound alpha chain similar to MHC class-1 molecules (Simister and Mostov, 1989). The function of FcRn receptors are to transport the immunoglobulin across the mammary epithelial barrier; however, the precise mechanism of transport is not yet known.

Colostrogenesis is regulated by lactogenic hormones. This distinct stage of mammary gland development is driven by estrogen and progesterone, which are necessary for initiation of IgG_1 transfer into colostrum (Barrington et al., 2001). Specifically, a decrease in the ratio of

progesterone to estrogen late in gestation (caused by declining progesterone combined with rising estrogen levels) has been hypothesized to initiate IgG transfer in beef and dairy cows (Guy et al., 1994).

There are numerous documented nutritional factors in the prepartum period that have effects on colostrum synthesis. Well-fed dairy animals produce a larger volume of colostrum compared to underfed animals (Mellor and Murray, 1985). Conjugated linoleic acid administered in the dry period of dairy goats causes less of a decrease in blood plasma IgG concentration (6%) of the dam but has no effect on colostral IgG levels (Castro et. al., 2006). Selenium has been shown to increase colostrum IgG concentration in beef cows with late gestation supplementation (Awadeh et al., 1998).

Beyond nutritional factors, there are other factors that affect colostrogenesis. In cows, a higher lactation number is correlated with increased IgG colostral concentration (Oyeniyi and Hunter, 1978). In small ruminants, the effect of lactation number on colostral IgG concentration has had varying results in studies. Ha et al. (1986) showed that later lactation number goats had a higher colostrum IgG concentration. However, other studies did not observe a significant effect of lactation number on colostral IgG concentration (Dos Santos et al., 1994; Arügello et al. 2006; Kessler et al. 2019). Litter size also has unclear effects on IgG concentration in goats. One study reported that colostrum IgG was higher in mothers with twin goats than single goat kids (Csapó et al., 1994). On the other hand, Argüello et al. (2006) and Kessler et al. (2019) did not find a significant difference in IgG concentration dependent on litter size. Another factor that affects IgG concentration is gestation length. Arguello et al. (1999) found that goats with gestations of 146 days showed lower colostral IgG concentration than goats with a longer gestation period.

Kessler and colleagues (2019) examined variation of colostrum composition between goat breeds; colostral IgG concentrations varied between 4.8 and 75.0 mg/mL. Breeds included were Anglo-Nubian, Appenzell, Boer, Bunte Deutsche Edelziege, Chamois-colored, Grisons Striped, Peacock, Saanen, Toggenburg, and Valais Blackneck. Anglo-Nubian and Valais Blackneck breeds were classified as dual purpose goats for dairy and meat production. Appenzell, Bunte Deutsche Edelziege, Chamois-colored, Grisons Striped, Peacock, Saanen, and Toggenburg breeds were classified as dairy goats. The Boer breed was classified as a meat breed. Colostrum fat, protein, and lactose concentrations varied widely between goat breeds. In goats, fat, protein, and lactose concentrations were between 1.3 and 16.5%, 4.9 and 25.1%, and 2.1 and 6.0% respectively. Colostrum IgG concentration was measured via ELISA. The highest mean IgG concentration was found in the meat-type goats, Boer, (61.0 mg/mL). The lowest mean IgG concentration was found in milk-type goats, Bunte Deutsch Edelziege, (17.9 mg/mL). These data support the hypothesis that meat-type goats have higher colostral IgG concentration compared to milk-type breeds (Kessler et al., 2019). This is similar to the differences observed between beef and dairy cattle colostrum (Guy et al., 1994) and most likely due to differences in volume affecting concentration.

2.6. Methods of Colostrum Quality Evaluation

With the documented importance of feeding adequate colostrum, production managers must ensure the necessary volume and quality of colostrum is consumed in a timely manner. There are numerous methods to measure colostrum quality directly and indirectly. Radial immunodiffusion (RID), ELISA, and turbidimetric immunoassay (TIA) are direct methods to

quantify IgG in colostrum. Specific gravity, Brix, and color are indirect measurements that can be correlated to the previously mentioned examples of direct measurement to quantify IgG.

2.6.1. Specific gravity

Specific gravity is a rapid and inexpensive method used to estimate colostrum IgG concentration on the farm. This approach is utilized in the field for bovine colostrum since there is a documented linear relationship between colostral specific gravity and immunoglobulin concentration (Fleenor and Stott, 1980). A colostometer (hydrometer) for cattle was developed from these findings, which calibrated globulin concentration with color coded regions, and floated at an estimated concentration of IgG (Fleenor and Stott, 1980). Further research has demonstrated that this method is skewed by temperature (Mechor et al., 1991), breed, and season of the year for dairy cattle (Morin et al., 2001), limiting its practicality. In goats, it was found that the density of colostrum was temperature-dependent, limiting the applicability of the colostometer (Rudovsky et al., 2008). Additionally, the bovine colostometer requires far more volume than would be practical for evaluating goat colostrum.

2.6.2. Radial Immunodiffusion (RID)

Another method utilized to measure colostrum is RID, which is considered the gold standard for quantifying passive transfer by directly measuring immunoglobulin concentration in serum or immunoglobulin concentration in colostrum. The RID method requires minimal dilution and is therefore more often used to measure colostrum quality over the ELISA method. IgA, IgG, or IgM can all be measured via this method with specific antibodies. This technique involves placing an unknown amount of antigen (colostrum or serum) on a plate and allowing it

to diffuse radially from a well. The antibody-containing agar combines with the antigen, to reach a final area on precipitate. The antibody is species and immunoglobulin specific. This area can be quantified to determine the original immunoglobulin concentration (Mancini et al., 1965). Use of RID is time consuming, expensive, and tedious so it is rarely used in on-farm settings (Quigley et al., 2013). The assay requires a laboratory environment, consistent procedure, trained technicians, and takes 24 hours to complete. Levieux (2002) found the IgG colostral concentrations were 47.9±25.5 mg/mL in goats of Saanen and Alpine breeds when measured via RID.

2.6.3. Enzyme-linked immunosorbent assay (ELISA)

ELISA is another method that measures colostrum IgG concentration and can also be used to measure serum IgG concentration. This method is most often used for serum due to the extensive dilution required to measure colostrum with ELISA. Generally, the ELISA is a plate-based assay designed to quantify a variety of substances ranging from peptides to hormones. The principle behind the method relies on an antigen binding to a solid surface and then washing away materials that are not bound. After, the bound materials complex with an antibody that is linked to an enzyme, commonly horseradish peroxidase or alkaline phosphatase. Detection is determined by assessing the conjugated enzyme activity via incubation with a substrate to produce a measurable product. The antibody-antigen interaction is highly specific and is important in the sensitivity of the test ("Overview of ELISA," Thermo Fisher Scientific).

Specifically with the goat ELISA, the principle method relies on microplate wells coated with polyclonal antibodies to goat IgG. This comprises the capture phase of the assay in which antibodies bind uniformly to goat IgG in the sample. The captured goat IgG then reacts with a

detector antibody. The detector antibody is a polyclonal anti-goat IgG (conjugated with horseradish peroxidase). Enzyme activity in the wells is quantified using tetramethyl benzide as a substrate and measured with a microplate reader (Amendola, 2019). In the previous studies, goat colostrum samples were measured via ELISA by Castro et al. (2018). Cow colostrum samples were measured via ELISA by Gelsinger et al. (2015).

2.6.4. Brix Refractometer

Brix percentage is a measure of sucrose concentration and was originally used for liquids such as wine, maple syrup, and fruit juice. More recently, the Brix has been found to work in non-sucrose containing liquids by approximating total solids percentage of colostrum or serum, which has been related to IgG in several studies. There are two different models of the Brix refractometer. In an optical model, the refractometer is held up to a light source and the Brix value read on the scale. In a digital model, a button is pressed to read the Brix percentage (Heinrichs and Jones, 2016). The Brix refractometer has been evaluated as a method to estimate IgG concentration in horses (Charvatte et al., 1998; Cash, 1999), sheep (Harker, 1978), cattle (Chigerwe et al., 2008; Bielmann et al., 2010; Morrill et al., 2012), and goats (Castro et al., 2018).

The Brix refractometer may be the most widely used method to measure colostrum quality on farm because it is inexpensive, readily available, and hardy (Quigley et al., 2013). To use a Brix refractometer, a few drops of colostrum are placed on the prism and the cover lowered.

2.6.5. Color

Another method used for colostrum quality estimation in goat and dairy cows is the color method. Argüello et. al. (2005) found a significant linear relationship between the color method and IgG concentration in goat colostrum, as confirmed with RID technique (r^2=0.695). The color method involves comparing the fresh colostrum to Chroma-meter measurements. The Minolta CR200 Chromameter was used, which is an electronic color analyzer that measures L* (relative lightness), a* (relative redness), and b* (relative yellowness). A chroma value was calculated using Chroma=(.5(a* + b*)) and correlated with IgG measured via RID. Despite the success of the study, it is not widely used or accepted as a method to measure colostrum quality in goats.

2.6.7. Automated Turbidimetric Immunoassay

The Turbidimetric Immunoassay (TIA) is based on immunologic agglutination and light scattering of the agglutination products. Specifically, antibodies are directed at a specific antigen to form immune complexes such as IgG. The number of immune complexes produced are proportional to the concentration of IgG in the sample. This is measured via turbidity with a calibrated spectrophotometer (Ferris and McCue, 2009). An automated TIA can run shorter than RID. Furthermore, since the TIA is automated, there is with a decreased likelihood of human error compared to the in RID with the measuring the precipitin rings (Davis et. al., 2005).

2.6.8. Method Comparison

Castro et al. (2018) reported a relationship between IgG concentration of goat colostrum measured by ELISA and a clinical refractometer (r^2= 0.79). Dunn et al. (2017) reported multiple relationships between quality testing methods in dairy cow colostrum. The correlation between

colostrum IgG concentration measured by RID and ELISA was r_2= 0.83 ($p<.001$). The average IgG values determined via RID were 1.8 times greater than the values found using the ELISA method when assessing colostrum and calf sera IgG concentration. In other words, IgG concentrations were significantly lower when measured by the ELISA method compared to RID. Dunn et. al. (2007) found that the correlation between cow colostrum IgG concentration measured by ELISA and Brix was r_2=0.58 ($p<.001$). The correlation between colostrum IgG concentration measured by RID and Brix was poorer with an r_2 of 0.36. ($p=.005$). Bielmann et al. (2010) reported a stronger correlation of r_2=0.73 ($p<.001$) between Brix and RID values of colostral IgG in cattle. Davis et. al. (2005) compared the TIA to RID in equine colostrum and found a significant linear relationship r_2=.59 ($p<.0001$).

2.7. Colostrum Management

Colostrum is vital in the health of neonate ruminants and to ensure passive transfer, colostrum must be managed appropriately to prevent the immunoglobulins from being denatured. Therefore, it is important to invest time and resources into a comprehensive colostrum management plan.

2.7.1. Source

It is best to source colostrum from the individual dams on the farm. Pooled colostrum is not recommended because of the potential to spread diseases. In the event of a colostrum shortage, the source farm should be of similar or superior disease status. There are commercial colostrum replacement products of bovine origin that have unknown effectiveness in goats (Jansen and Menzies, 2015).

2.7.2. Collecting Colostrum

To collect colostrum, one should wear clean gloves and wipe teats and the udder with a clean cloth to prevent contamination. The teats should then be sanitized. The dam has not been milked during the dry-off period, so the pathogen load on the teat will be greater than a normal milking situation. Therefore, extra care should be given to ensure cleanliness. Pre-dip solution should be used on the teat, and the disinfecting solution should have contact time of at least 30 seconds to kill the pathogens (Bentley et al., 2017). The teat should then be wiped off with a dry, clean towel. Proper milking techniques should be utilized, and the colostrum should be collected within 15 minutes of kidding (Jansen and Menzies, 2015). All milking equipment should be cleaned and sanitized before and after every milking.

2.7.3. Storage

If colostrum is not fed immediately after collection, it can be stored in the refrigerator or freezer. Storing in the freezer preserves the colostrum for a longer time compared to the refrigerator. Colostrum can be frozen for six months and refrigerated for two to three days without damage to antibodies (Cullens, 2017). Cleanliness is key to storing colostrum because low-bacteria colostrum can be stored longer. Colostrum should be stored in clean, single serve containers (Cullens, 2017).

2.7.4. Effect of Freezing

Argüello et al. (2003) reported that one freeze-thaw cycle has little effect on IgG goat colostrum concentration when measured with the RID method. Repetition of freezing and thawing tended to reduce IgG concentration, but not to a significant degree. The method of

thawing did not affect the colostrum IgG concentration. Additionally, there were no observed significant effects of storing colostrum at 4 °C for three months. Morill et al. (2015) showed that in cow colostrum, IgG concentration was affected by multiple freeze thaw cycles with the RID measurement but unaffected in refractometry measurement. When measuring with the RID method, IgG concentration was found to be higher in fresh samples than after multiple freeze-thaw cycles. However, % Brix reading was unaffected by freezing and thawing (Morill, 2015). Freezing and thawing kills leukocytes, and there is evidence that these cells transfer immune information to the neonate (Van Amburgh, 2018).

2.7.5. Thawing

Colostrum that is frozen must be thawed and fed at body temperature (102-103°F). It is recommended to thaw in a warm water bath less than 120°F to prevent antibodies from being destroyed (Bentley, 2018).

2.7.6. Heat treatment of colostrum

Colostrum my contain pathogens as a result of shedding from the mammary gland, colostrum contamination after harvesting, or improper storage (McMartin et al., 2006). To reduce the incidence of disease, heat treatment and pasteurization are often utilized on farms. Control programs for caprine arthritis encephalitis (CAE) originally set the protocol of 56 °C for sixty minutes with the goal to inactivate the CAE virus without denaturing the immunoglobulins (Adams et al., 1983). It is recommended that a water bath or double boiler be used to heat treat colostrum since the temperature can be regulated more closely (Bentley, 2018).

There are contradicting reports on the effect of heat treatment on colostrum. Recently, in vitro studies have shown that following the protocol of heating the colostrum to 56 °C for sixty minutes reduces the measurable IgG concentration by 37% when measured with the RID method (Arguello et al., 2003). However, a study in calves found that heat treatment of colostrum at 60 °C for one hour resulted in higher concentrations of serum IgG and a greater absorption efficiency in heat-treated colostrum versus raw colostrum when measured with the RID method (Elizondo-Salazar and Heinrichs, 2009). Another study by Johnson et al. (2007) found that calves fed heat treated colostrum had a higher 24-h serum concentrations of total protein and IgG and a greater apparent efficiency of absorption versus raw colostrum. Total protein was measured with a handheld refractometer and IgG was measured via a turbidimetric immunoassay.

2.8. Passive Transfer

Passive transfer is a form of acquired immunity that is characterized by intact immunoglobulin absorption across the small intestine from colostrum directly after birth. Passive immunity in the neonate relies on the ability of intestinal cells to transport macromolecules from gut lumen into the bloodstream. Immediately after birth, intestinal uptake is nonselective and transient in cattle, sheep, goats, and swine (Hurley and Theil, 2011). This species-specific unique uptake is controlled by nutrient independent factors present in colostrum. The ability of a farm-animal to absorb immunoglobulins decreases a day or two after birth until the intestine no longer absorbs macromolecules in a process deemed intestinal closure (Pond et al., 2011). In goat kids, peak IgG concentration was observed 24 hours after birth (Argüello et al., 2004).

Specifically, colostral absorption occurs in small intestinal cells via the neonatal receptor FcRn, which transports IgG across the intestinal epithelium via pinocytosis (Israel et al., 1995). Neonatal corticosteroid levels must be high for effective colostral absorption and factors such as premature birth and cesarean sections may inhibit cortisol release and therefore decrease colostral absorption (Sangild, 2003).

Failure of passive transfer occurs when the threshold concentration of IgG for a specific species is not reached before closure occurs, either via inadequate colostrum quantity or quality within the first hours of life. The threshold concentration of IgG is the minimum amount of IgG necessary in sera to ensure the vitality of the neonate, which is determined experimentally for each individual species.

Passive transfer in kid goats is measured through serum immunoglobulin levels. In one study (O' Brien and Sherman, 1993), kids were fed their individual dam's colostrum and the mean serum IgG level varied from kid to kid. The mean serum immunoglobulin level for all kids was 11.70 mg/mL. The mean serum concentration for healthy kids was 14.39 mg/mL. The mean serum IgG level for treated kids was 7.06 mg/mL and the mean for dead kids was 7.50 mg/mL. A failure of passive transfer was determined to occur when the circulating IgG levels are less than 12.00 mg/mL. In this study 47% of kids below the mean serum immunoglobulin levels died, while only 13.6% of kids above the mean died. Overall, this study showed a significantly higher mean serum IgG level in kids that survived to weaning versus those that died and confirmed that kid health and survival is associated with passive transfer of immunoglobulins. From this study, a recommendation of a minimum of 12.00 mg/mL of serum IgG is required in newborn kids for vitality. In calves, a recommendation of a minimum of 10.00 mg/mL of serum IgG is required for calf vitality (Elizondo-Salazar and Heinrichs, 2009).

To ensure passive transfer, it is recommended that kids receive 10% of their body weight (BW) of good quality colostrum, also measured as more than 3.0 g IgG/kg BW (Constant et al., 1994) in serum. Castro et al. (2007) determined 4 g IgG/kg BW in serum within 24 h provided adequate passive transfer [according to O'Brien and Sherman's (1993) definition] for 80% of the kids studied. Bentley (2018) recommended a minimum colostrum volume intake of 10% BW.

Passive transfer is affected by volume of colostrum fed, colostral IgG concentration, and total amount of immunoglobulin fed (Stott and Fellah, 1983). In calves, Stott and Fellah (1983) found that IgG colostrum concentration is more important than the quantity of the colostrum fed if total consumed IgG was the same. Calves that consumed the higher concentration of colostrum at a lower volume had higher levels of IgG in the blood. In goat kids, Argüello et al. (2004) showed that quantity of IgG consumed had a positive high correlation with IgG blood concentrations within the first 72 hours of life. Michanek and Ventorp (1989) explored the effect of age at first feeding on passive transfer. They found that calves fed colostrum at one hour old absorbed more colostrum in later feedings compared to other first feeding times.

2.9. Methods of Evaluation of Passive Transfer

2.9.1. Enzyme-linked immunosorbent assay (ELISA)

The ELISA method can be used to measure immunoglobulins in colostrum as discussed earlier and also to measure immunoglobulins in serum as a method to evaluate passive transfer. ELISA is utilized more often in serum since extensive dilutions are required for colostrum with this method. The principle of the ELISA method involves antibody-antigen interaction and is the same regardless of measuring immunoglobulin in colostrum or serum. The IgG concentration of

goat serum samples were measured via ELISA by Rodríguez et al. (2009). The IgG concentration of cow plasma was measured via ELISA by (Gelsinger et al., 2015).

2.9.2. Total Protein

Measuring total protein of serum can be used to evaluate passive transfer of IgG. Serum total protein from neonates can be evaluated via a refractometer tool. Studies have shown refractometry serum total protein concentration is closely correlated with serum IgG concentration in lambs ($P < 0.001$; $r_2 = 0.848$) (Massimini et al., 2006). Therefore, it has been suggested as a valuable method to measure passive transfer status. In calves, a serum total protein level of 5.0 g/dL predicts a serum IgG level of 10 mg/mL, which is indicative of successful passive transfer (Swan et al., 2007). Another study found that a serum protein concentration of 5.2 g/dL was equivalent to 10 mg/mL serum IgG_1 in calves (Tyler et al., 1996). There is minimal data available regarding total protein and passive transfer in goats.

2.9.3 Zinc Sulfate Turbidity Test

The zinc sulfate turbidity test is used in the lab to evaluate total immunoglobulin (not specifically IgG) quantity in serum. The principle behind the test relies on selective precipitation of high molecular weight proteins, such as immunoglobins, due to a reaction with a salt. This test is not widely used due its poor specificity (Constable et al., 2016).

2.9.4. Method Comparison

Dunn et al. (2017) reported multiple relationships between quality testing methods in dairy cow serum. The correlation between calf sera IgG concentration measured by RID and

ELISA was r_2=.97 ($p<.001$). The average IgG values determined via RID were 1.8 times greater than the values found using the ELISA method when assessing colostrum and calf sera IgG concentration. In other words, IgG concentrations were significantly lower when measured by the ELISA method compared to RID. The RID and ELISA methods had a significant relationship with Zinc Sulphate Turbidity Test ($p<.001$) with correlations of r_2=.78 and r_2=0.77 respectively.

2.10. Evaluation of Passive Transfer

Although the correlation between serum IgG and kid health has been documented, there are varying results regarding colostrum consumed and serum IgG concentration. In one study, Dos Santos et al. (1994) did not find a relationship between consumed IgG concentration and serum IgG concentration in one day old kids. In calves, however, it has been observed that those fed a higher concentrated colostrum will have greater IgG absorption (Castro et al., 2005). Constant et al. (1994) showed the effect of colostrum concentration and volume on serum IgG levels in kid goats. The kids fed a higher dose colostrum (3.0g of IgG/kg) had a higher serum IgG concentration of 17.27 ± 2.44 mg/mL compared to those fed a lower dose of colostrum (1.5 IgG/kg) with a concentration of 14.60 ± 2.88 mg/mL. The mean peak IgG concentration was 17% higher in kids fed the higher dosage than kids fed the lower dosage.

Rodríguez et al. (2009) evaluated the effect of IgG concentration on goat immune status when total amount of IgG fed was constant. All kids received 4 g of IgG per kg of bodyweight but were fed in groups of varying concentration (20, 40, 60, and 80 mg/mL). Blood samples were obtained on days 0-5 postpartum and serum immunoglobulin concentration was measured via the

ELISA method. The plasma IgG and IgM concentrations were highest on day one in the kids fed colostrum at 80 mg/mL.

Rodriguez et al. (2009) calculated the apparent efficiency of absorption (AEA) in kid goats as [plasma IgG (mg/mL at d 1) × plasma volume (mL)/IgG intake (mg)] × 100, which measures the proportion of total IgG mass fed that is absorbed into the animals' circulation. Plasma volume was determined to be 9.9% of birth weight from a study by Quigley et al. (1998) on calves. Rodriguez et al. (2009) calculated the apparent efficiency of absorption to be 13.3, 13.3, 14.9, and 24.4% in groups of IgG concentration of 20 mg/mL, 40 mg/mL, 60 mg/mL, and 80 mg/mL respectively. The AEA was higher in the 80 mg/ ml group (24.4%) compared to the other groups of lower colostrum concentration. Increasing the immunoglobulin concentration in colostrum improves immunoglobulin absorption at the same amount of total immunoglobulin fed. Johnson et. al. (2007) calculated AEA in calves to be around 25%, which is similar to that of kids fed at the highest IgG concentration in Rodriguez's study. However, natural goat colostrum does not have as high of immunoglobulin concentration, so it is estimated at AEA in goats would be lower.

Chen et al. (1999) found significant differences in serum IgG concentrations when comparing kids fed colostrum with high or low protein concentration (20 g/ dL and 10 g/dL). Higher colostrum protein consumption is correlated with higher serum IgG concentration in kid serum. The overall mean serum total protein and gamma globulin concentrations were low at birth (9.40 and 0.13 g/dl). The levels peaked around 24 h (12.45 and 3.33 g/dl) and remained relatively constant with slight decrease through 5 days (11.50 and 2.23 g/dl). The serum total protein and IgG concentration was measured via a colorimetric assay and an agarose gel electrophoresis kit.

Another study was completed by Furman-Fratczak et al. (2011) involving dairy heifer calves. Serum immunoglobulin concentration was measured via RID in the calves between 30 and 60 hours of life and then they were divided into four groups (immunoglobulin <5, 5-10, 10-15, and >15 mg/mL). The disease rate was lowest in the groups of immunoglobulin concentration greater than 10 mg/mL, and calves with >15 mg/mL immunoglobulin did not have any incidence of respiratory tract infections. The quality of the ingested colostrum was determined via a colostrometer and categorized as bad (<39 mg of immunoglobulin/ml), passing (42–77 mg of immunoglobulin/ml), good (80–118 mg of immunoglobulin/ml), or very good (>121 mg of immunoglobulin/ml). The mean colostrum immunoglobulin concentration (63.2 mg/mL) was lowest in calves with immunoglobulin between 5 and 10 mg/mL. The mean colostrum concentration (104.8 mg/mL) was highest in calves with immunoglobulin >15mg/mL. Therefore, the quality of colostrum consumed was related to the quantity of immunoglobulin in serum. Long-lasting impacts of adequate passive transfer are illustrated by the finding that calves with immunoglobulin >10 mg/mL at 30-60 hours of life reached body weights allowing for first insemination sooner (Furman-Fratczak et al., 2011).

Total protein of neonate serum is a proposed method to evaluate passive transfer. Tyler et al. (1996) found that a serum protein concentration of 5.2 g/dL was equivalent to 10 mg/mL serum IgG_1 in calves. Ballou (2012) found that total serum protein and frequency of failure of passive transfer varied between breeds of dairy cattle ($p<0.01$). A failure of passive transfer (serum total protein <5.2 g/dL) occurred in 62.5% of Holstein and 26.3% of Jersey calves. The average total protein for Holstein calves was 4.9 g/dL and the total protein for Jersey calves ranged from 5.8 to 5.9 g/dL. There are minimal data regarding total protein and passive transfer in goats.

Chapter 3

USE OF BRIX REFRACTOMETER IN ASSESSING GOAT COLOSTRUM

3.1. Abstract

Feeding high-quality colostrum is the single most important event in the newborn kid's life to ensure viability and survivability. Quality is based on the concentration of immunoglobulin G (IgG) in colostrum. Dairy cattle colostrum quality assessment with a Brix refractometer has been validated (Bielmann et al., 2010). A practical application of using a Brix refractometer for testing goat colostrum would be useful if a validated method existed. A total of 114 paired Brix and radial immunodiffusion (RID) determinations were performed on 58 individual and 56 pooled doe colostrum samples. Colostrum quality declined exponentially with time from kidding (IgG, mg/ml = $97.9*e^{(-0.05t)}$, r^2=0.35, P<0.0001) but was highly variable. Mean ± standard deviation (median, range) RID concentrations for all samples was 71.0 ± 36.8 mg/mL (74.2, 4.2-180.5). Overall post-thaw Brix determinations were 20.7 ± 4.5 (20.3, 8.7-34.3). All Brix and RID determinations were normally distributed. Brix measurements determined in fresh and thawed colostrum where highly correlated (P<0.0001) for all (r= 0.97), individual (r=0.98) and pooled (r=0.76) samples. Similarly, post-thaw Brix and RID measures were highly correlated (P<0.0001) in overall (r=0.85), individual (r=0.89) and pooled (r=0.77) samples. Brix determinations on individual samples were influenced by lactation number (P=0.084), but not dry period length or number of kids. The overall prediction model relating Brix to RID in goat colostrum (Figure 1) was RID (mg/mL) = 6.97(Brix)-73.65 (r^2=0.73, P<0.0001). These data suggest the digital Brix refractometer is predictive of colostrum IgG concentration for goats, as it is for dairy cattle colostrum. In comparison to the reported association in dairy cattle, colostrum containing 50 mg/ml IgG had a Brix value of 18 in goats compared to 22 in cattle (Bielmann et

al., 2010). Brix measures were not different between fresh and post-thaw samples (r_2=0.94, P<0.0001). No differences in Brix or IgG concentration with heat treatment suggests no degradation in IgG or inadequate heat treatment. Further research to determine desired colostrum IgG concentration for successful passive transfer is needed.

3.2. Introduction

Colostrum is the unique mammary secretion in all mammals defined as the first milk produced immediately following parturition. Colostrogenesis is the transfer of immunoglobulins from the blood to colostrum in the mother. This transfer of immunoglobulins from dam serum to milk occurs so that the milk has immune-boosting components for immunodeficient young. At birth, ruminants are agammaglobulinemic due to the inability of uterine and fetal tissues to transfer maternal immunoglobulin molecules (Halliday and Williams, 1979). Most farm animal species, including goat kids, rely on intestinal absorption of colostral immunoglobulins to provide passive humoral immunity, and stimulation of other aspects of immune responses initiated by maternal lymphocytes and other immune regulators (Van Amburgh, 2018).

Feeding of high-quality colostrum is the single most important event in life of a newborn ruminant to ensure viability and survivability. Colostrum quality is based on the concentration of IgG. Numerous studies have documented the importance of colostrum and successful passive transfer of immunoglobulins for health, growth, and survival of animals. Failure of passive transfer occurs when not enough antibodies are absorbed after birth. Failure of passive transfer is the primary cause of increased susceptibility to infectious disease, increased morbidity, and increased mortality rates in newborn kids (O'Brien et al, 1993). Therefore, high-quality colostrum is essential for kid health.

In calves, there are long-term benefits documented with adequate colostrum intake such as improved rate of gain and feed efficiency (Robison et al., 1988), reduced age at first calving, and improved milk production (DeNise et al.,1989). In goats, similar long-term benefits have been documented showing increased rate of gain in relation to elevated serum IgG concentrations 24 hours after parturition (Massimini et al., 2007).

With the documented importance of feeding adequate colostrum, production managers must ensure the necessary volume and quality of colostrum is consumed in a timely manner. There are numerous methods to measure colostrum quality directly and indirectly. Radial immunodiffusion (RID) and enzyme-linked immunosorbent assay (ELISA) are direct methods to quantify IgG in colostrum. Specific gravity and Brix are indirect measurements that can be correlated to direct measurement to quantify IgG.

Specific gravity analysis, using a colostrometer, is a rapid and inexpensive method used to estimate colostrum IgG concentration on the farm. In goats, it was found that the density of colostrum was temperature-dependent, limiting the applicability of the colostometer (Rudovsky et al., 2008). Additionally, a high volume of colostrum is necessary to float the colostrometer, which limits the application of specific gravity as a method to measure IgG in goats since they have lower colostrum volume.

Another method utilized to measure colostrum quality is RID, which is considered the gold standard for measuring immunoglobulin concentration colostrum. RID is time consuming, expensive, and tedious, so it is rarely used in on-farm settings (Quigley et al., 2013). The assay requires a laboratory environment, consistent procedure, trained technicians, and takes 24 hours to complete.

ELISA is another method that measures colostrum IgG concentration and can also be used to measure serum IgG concentration. This method is most often used for serum due to the extensive dilution required to measure colostrum with ELISA.

The Brix refractometer may be the most widely used method to measure colostrum quality on farm because it is inexpensive, readily available, and hardy (Quigley et al., 2013). Originally, Brix percentage (a measure of sucrose concentration) was used for liquids such as wine, maple syrup, and fruit juice. More recently, the Brix has been found to work in non-sucrose containing liquids by approximating total solids percentage of colostrum or serum, which has been related to IgG in several studies. The Brix refractometer has been evaluated as a method to estimate IgG concentration in horses (Charvatte et al., 1998; Cash, 1999), sheep (Harker, 1978), cattle (Chigerwe et al., 2008; Bielmann et al., 2010; Morrill et al., 2012), and goats (Castro et al., 2018).

Dairy cattle colostrum quality assessment with a Brix refractometer has been validated (Bielmann et al., 2010). A practical application of using a Brix refractometer for testing goat colostrum would be useful if a valid method.

The objective of this study was to determine if there exists a relationship between Brix reading and IgG concentration in goat colostrum similar to what has been documented in dairy cattle.

3.3. Materials and Methods

All procedures were reviewed and approved by the Pennsylvania State University Institutional Animal Care and Use Committee (IACUC#: 47927).

3.3.1. Housing, Animals, and Feeding

A commercial, Humane-Certified, 900-doe goat dairy located in Humboldt County, California was the source for sample collection during 2018. Local veterinary service is used for routine health care and a consulting veterinarian provides consultation on herd health management, reproductive assessment and nutritional formulation. Goats are housed in three large greenhouse barns with four group pens capable of loosely housing 100-150 goats per pen. Most pens were maintained at 65-85% of capacity as the dairy was continuing to increase herd size. The dairy used extended lactations and out-of-season breeding to maintain a milk supply to the associated processing plant year-round. Loose housing areas were bedded with locally sourced almond shells.

Animal grouping was based on production stage and varied with percent of herd in lactation. Production groups included extended lactation (>300 days in milk), early lactation (<40 DIM), and high production (>40 DIM). Dry does were divided into a 2-group system consisting of far-off (< 30 days prior to kidding) and close-up (>30 days prior to kidding) does. Pregnant doelings were placed into the close-up pen at least 60 days prior to kidding. After kidding does were placed in a post-partum pen for a few days before being moved into the early lactation group. Kids were fed colostrum and moved to a separate kid barn and fed milk replacer and starter grain.

Dairy goat breeds represented on the farm included Toggenburg, Saanen, Alpine, and Lamancha. There was no separation by breed and all does were intermixed. Most does were crossbreds of two or more of these breeds. Farm management had an electronic herd record system as well as a parlor that provided daily milk weights. All data relative to doe age, kidding date, kid numbers, live vs. dead, birth weights, time from kidding to sampling and lactation

number were compiled. Data relative to days dry, previous lactation length and production were also collected.

Lactating pens were fed a total mixed ration (TMR) twice daily. Far-off dry and close-up dry pens were fed the TMR that was top-dressed with additional grains (Table 2.). Feed ingredients and representative samples of the TMR were analyzed for nutrient composition (Table 3) at a commercial laboratory (Cumberland Valley Analytical Services, Waynesboro, PA).

Table 2. Ingredient composition of TMR, grain mix, and custom pellet supplement fed to the goat herd.

Ingredient	TMR Mix[1]	Grain Mix	Pellet Supplement
Alfalfa-grass hay	30.5		
Mixed mostly grass hay	16.7		
Beet pulp pellets	5.8		
Almond hulls	19.4		
Grain mix	10.2		
Pellet Supplement	17.4	47.6	
Corn, rolled		21.9	
Barley, rolled		11.0	
Beet pulp-shreds		14.8	
Molasses		4.7	10.0
Soybean meal			36.5
Soyhulls			25.0
Mineral premix			17.5
Sodium Bicarbonate			5.0
Magnesium oxide			1.0
Yeast			5.0

Table 2 shows the ingredient composition of the TMR, grain mix, and custom pellet supplementation that was fed to the goat herd. Lactating pens were fed TMR twice daily, and far-off dry and close-up dry pens were fed the TMR that was top-dressed with additional grains,

[1]Base TMR blend to which an equal blend of grain mix, beet pulp pellets and pellet supplement were top-dressed. [Unit (%)]

Table 3. Nutrient composition (DM basis) of feed ingredients in goat diets.

Nutrient[1]	Alfalfa Hay	MMG Hay	Pellet Supplement	Almond Hulls
Dry matter, %	89.8	88.8	88.1	88.3
Crude protein, %	19.8	16.3	17.7	4.9
NDF, %	42.9	50.7	26.6	20.2
ADF, %	32.2	33.4	14.5	11.7
Starch, %			28.9	0.8
NFC, %	20.4	37.1	47.3	68.2
Calcium, %	1.0	1.01	0.91	0.22
Phosphorus, %	0.29	0.32	0.49	0.10
Magnesium, %	0.43	0.31	0.63	0.12
Potassium, %	3.1	1.67	1.16	2.56
Sodium, %	0.09	0.31	0.98	0.02
Iron, ppm	543		223	287
Copper, ppm	8		42	5
Manganese, ppm	30		122	16
Zinc, ppm	17		196	11

Table 3 shows the analyzed nutrient composition of the feed ingredients. These ingredients were analyzed at Cumberland Valley Analytical Services.
[1]Abbreviations: NDF: neutral detergent fiber; ADF: acid detergent fiber; NFC: non fiber carbohydrates, calculated value

3.3.2. Colostrum Sampling

Colostrum sampling was completed by trained farm staff over a period between January 15 and April 12, 2018. Colostrum samples were collected as close to parturition as possible. A standard operating procedure for proper colostrum sampling to minimize potential bacterial contamination was developed and provided to all farm personnel involved in sample collection. The farm protocol for colostrum management was to collect fresh samples from does and then pool between 3 and 6 doe samples for heat treatment. Only heat-treated pooled samples were fed to female kids. Individual doe samples were collected as part of the study and this colostrum was only fed to male kids. Colostrum was heated to 135 °F (60 °C) for 1 hour. Colostrum samples were collected into 50 ml plastic vials labeled with a goat or pool identification number and

collection date, frozen (-20°C) and shipped on ice to the Central Milk Testing Laboratory (CMTL) at Pennsylvania State University. Information as to the does included in a pooled sample were provided.

3.3.3. Colostrum Measurements

Colostrum immunoglobulin content was evaluated with a Brix refractometer (Palm Abby, MISCO, Solon, OH) and quantified by RID using a commercial goat-based assay kit (Triple-J Farms, Bellingham, WA).

On farm digital Brix determinations were performed in duplicate on the fresh individual doe and pooled colostrum samples. Pooled samples were also evaluated following heat treatment. A subsample of fresh and pooled (before and after heat treatment) colostrum was frozen and shipped to Penn State University for a post-thaw Brix determinations and measurement of total IgG concentration using RID (Triple-J Farms, Bellingham, WA). Before measurements, colostrum samples were allowed to reach room temperature by either using a hot water bath set to 50° C or by sitting on the bench top.

3.3.4. Brix Reading

After reaching room temperature, the colostrum sample was inverted at least twice. The refractometer was calibrated with a drop of distilled water. A drop of colostrum from a pipette was transferred to the prism of the refractometer. The sample cover was lowered, and the sample read on “Brix” mode. The refractometer was washed with distilled water and paper towel between readings. This measurement was completed in triplicate for each sample.

3.3.5. Radial Immunodiffusion (RID)

The RID method was preformed according to the manufacturer's instructions for Goat IgG RID Kits produced by Triple J Farm. Depending on the Brix reading, different dilution procedures were carried out. Each sample was run in duplicate.

To dilute the colostrum prior to testing, each sample tube was inverted at least twice. Prior to dilution, tubes were filled with the appropriate amount of saline based on the Brix measurement. For a samples with a Brix reading > 19, 1.8 mL tubes were filled with 900 microliters of sterile saline. For samples with a Brix reading < 20, 1.8 mL tubes were filled with 800 microliters of sterile saline. Colostrum was added to the dilution tubes; 100 uL of colostrum were pipetted into appropriate tubes for samples that have a Brix reading > 19 and 200 microliters were pipetted into tubes for Brix readings < 20. A new pipette tip was used for each sample. To help reduce dilution errors, pipette tips were wetted, colostrum drawn up slowly (since colostrum is often quite viscous), and pipette tips were wiped with a paper towel after drawing up and before depositing sample into dilution tube. After each sample was diluted, the dilution tubes were vortexed for three seconds.

After dilution, 5 microliters of each sample or standard was pipetted to the bottom of each well. The lid of the plate was closed and placed right side up in a foil Ziploc bag (included in kit). The plate was set on the countertop at room temperature for 48 hours. After 48 hours, thee diffused rings (in mm) around each well was read at exactly 48 hours. Samples with a coefficient of variation greater than 10 were repeated in accordance with guidelines set specifically for this study.

3.3.6. Statistical Analysis

Colostral Brix and RID data were tested for normality using Proc UNIVARIATE in SAS (ver. 9.4, Carey, NC). Both Brix and RID determinations of colostrum IgG content were analyzed by ANOVA (Proc GLM) to determine the influence of sample source (farm or lab), type (individual or pooled) and heat treatment. Relationship between colostrum Brix value and IgG concentration determined by RID was established using correlation analysis (Proc CORR) and further explored with ANOVA where the model included various sample parameters as covariants. A predictive relationship between Brix and RID was determined using regression modeling (Proc REG). Once a regression model was established, Brix variables were converted to predicted IgG concentration and compared to RID values using Bland-Altman plots. Statistical significance was assigned at $P \leq 0.05$ unless otherwise indicated.

3.4. Results

A total 114 paired Brix and RID determinations were performed on 58 individual and 56 pooled doe colostrum samples. These samples were all post-thaw while 58 Brix determinations were made on fresh colostrum samples. Brix and RID determinations were tested for normality using univariate procedure and were normally distributed.

3.4.1. Averages and Correlation Coefficients

Fresh colostrum samples were collected 6.4 ± 7.3 h post kidding (median: 3.25; range: 0-26.5 h). Colostrum quality declined exponentially with time from kidding (IgG, mg/ml = $97.9*e^{(-0.05t)}$, r^2=0.35, P<0.0001) but was highly variable. Mean ± standard deviation (median, range) RID concentrations for all samples was 71.0 ± 36.8 mg/mL (74.2, 4.2-180.5). Overall post-thaw

Brix determinations were 20.7 ± 4.5 (20.3, 8.7-34.3). Fresh sample mean Brix values were 21.2 ± 4.7 (21.7, 9.1-34.0). Brix measurements determined in fresh and thawed colostrum where highly correlated ($P<0.0001$) for all ($r= 0.97$), individual ($r=0.98$) or pooled ($r=0.76$) samples. Similarly, post-thaw Brix and RID measures were highly correlated ($P<0.0001$) in overall ($r=0.85$), individual ($r=0.89$) and pooled ($r=0.77$) samples. Accounting for type of sample (individual vs. pooled) influenced ($P=0.045$) the association between Brix in fresh and post-thaw samples ($r^2=0.94$, $P<0.0001$). Brix determinations on individual samples tended to be influenced by lactation number ($P=0.084$), but not dry period length or number of kids. The average Brix before heat treatment of a subset of pooled samples was 20.51 and the average Brix after heat treatment was 20.63. The average IgG concentration before heat treatment of the subset of pooled samples was 67.69 mg/ml, and the average IgG concentration of the pooled samples after heat treatment was 67.86 mg/ml. Heating did not influence the comparisons of Brix fresh sample versus post thaw Brix on the same samples ($P=.8127$). Overall prediction model relating Brix to RID in goat colostrum was RID (mg/mL)=6.97(Brix)-73.65 ($r^2=0.73$, $P<0.0001$). The scatter plots and regression analysis for Brix and RID are represented in Figure 1.

Figure 1. IgG (mg/ml) vs. Brix

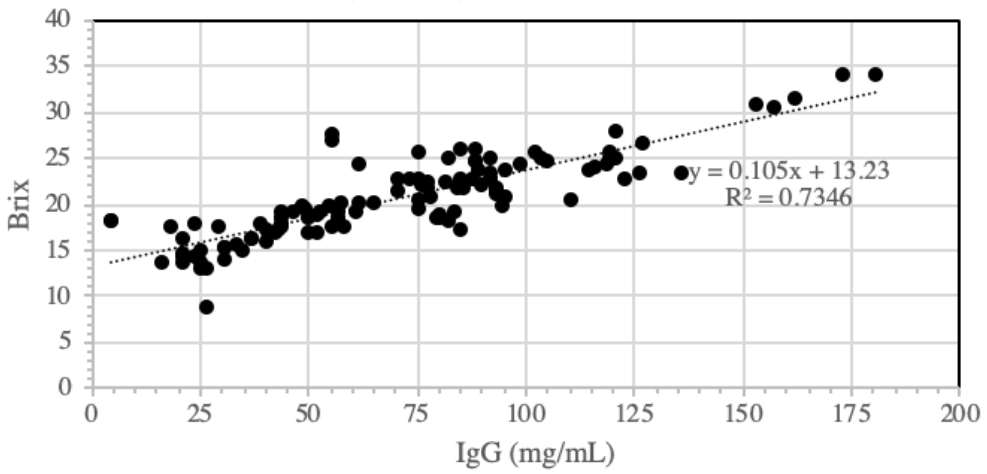

Figure 1. shows the overall regression of Brix determination on colostrum IgG determination (via RID) for 114 goat colostrum samples including individual (fresh and post-thaw) and pooled (fresh, post-thaw, and post-heated).

3.4.2. Test Characteristics

The digital Brix refractometer was analyzed for test characteristics against the RID test. A positive sample was defined as containing IgG concentration above the cut-off point on the RID test. Cut-off points for RID were defined at 50 mg/ml, 60 mg/ml, and 79 mg/ml. Brix cut-off values were evaluated between 17 and 22, with the most relevant values listed in Table 3. The calculated sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were used to identify the appropriate cut-off points. The highest values for 50 mg/ml were found to be at the 18 Brix cut-off level with the sensitivity, specificity, PPV, and NPV calculated to be 89.74%, 90.48%, 94.59%, and 82.61% respectively. The highest values for 60 mg/ml were found to be at the 19 Brix cut-off level with the sensitivity, specificity, PPV, and NPV calculated to be 90.32%, 82.76%, 84.85%, and 88.89% respectively. The highest values for 79 mg/ml were found to be at the 21 Brix cut-off level with the sensitivity, specificity, PPV, and NPV calculated to be 80.00%, 88.57%, 83.33%, and 86.11% respectively.

Table 4. Diagnostic test characteristics for measuring fresh colostrum with Brix compared with RID

RID	Brix	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
50 mg/ml	17	100	80.95	90.70	100
	18	89.74	90.48	94.59	82.61
	19	82.05	95.24	96.97	74.07
60 mg/ml	17	100	58.62	72.09	100
	19	90.32	82.76	84.85	88.89
	21	70.97	93.10	91.67	75
79 mg/ml	20	80.00	84.38	71.43	77.14
	21	80.00	88.57	83.33	86.11
	22	68.00	80.95	88.57	79.49

Table 4. shows the diagnostic test characteristics for measuring fresh colostrum with Brix compared with RID. The highest values for 50, 60, and 79 (mg/mL) were found to be at the 18, 19, and 21 Brix cut-off level respectively.
PPV=positive predicted value, NPV=negative predicted value

3.4.3. Bland-Altmann

The Brix variables were converted to predicted IgG concentration with the experimental regression from this study (RID (mg/mL)=6.97(Brix)-73.65) and the Bielmann et al. (2010) study and then compared to RID values using Bland-Altman plots. In our model (Figure 2), the mean was 0 and the limits of agreement were (-37.73-37.73) in the Bland-Altman Plot. In the Bielmann model (Figure 3) the mean was -36.69 and the limits of agreement were (-86.88 - 13.50).

Figure 2. Bland-Altman Plot (our model)

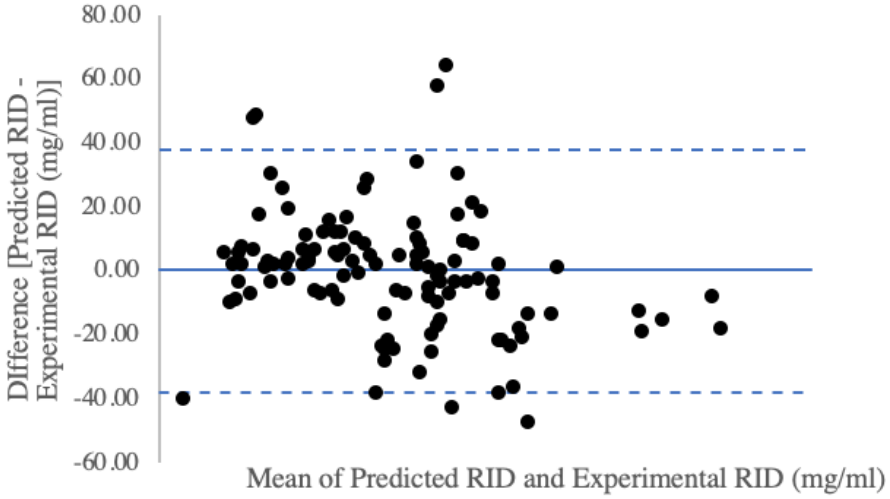

Figure 2. Bland- Altman plot of the difference in IgG concentration predicted by regression (Figure 1.) and the experimental measured RID value of goat colostrum.

Figure 3. Bland-Altman Plot (Bielmann model)

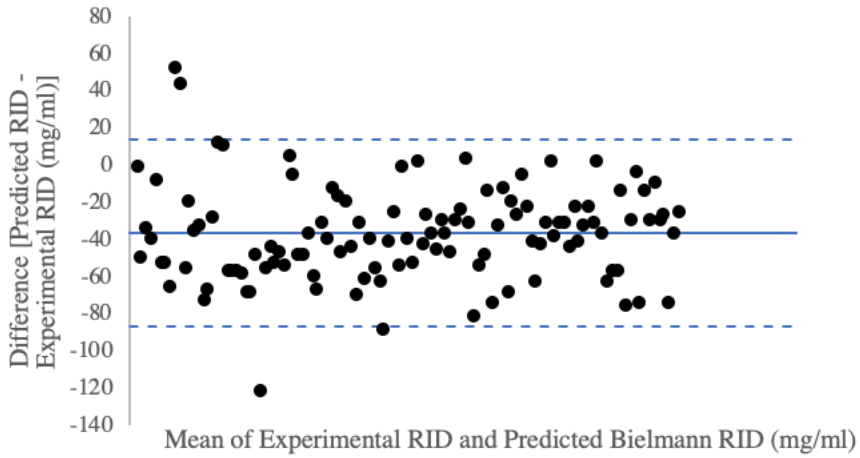

Figure 3. Bland-Altman plot of the difference in IgG concentration predicted by the Bielmann et al. (2010) regression from cattle and the experimental measured RID value of goat colostrum.

3.5. Discussion

3.5.1. RID Values-Methodology Differences

Mean ± standard deviation (median, range) RID concentrations for all samples was 71.0 ± 36.8 mg/mL (74.2, 4.2-180.5). Quigley et al. (2013) reported a similar value in cattle of 73.4 ± 26.2 mg/ml for the average colostrum IgG concentration measured by RID. However, other researchers evaluating colostrum IgG concentration in goats have found lower values, which may be attributed to methodology differences, breed, or nutritional differences. Both Rudovsky et al. (2008) and Romero et al. (2013) found the average colostrum IgG concentration in goats measured by ELISA to be lower, 49.1 mg/ml and 28.23 mg/ml, respectively. Dunn et al. (2017) directly compared RID and ELISA methods in cow colostrum and found that the average IgG values determined via RID were 1.8 times greater than the values found using the ELISA method when assessing colostrum. In other words, IgG concentrations were significantly lower when measured by the ELISA method compared to RID. Gelsinger et al. (2015) also found a consistent underestimation of IgG concentration by ELISA compared with RID when evaluating cattle colostrum quality.

Most recently, Batmaz et al. (2019) measured the mean IgG concentration of first milking goat colostrum samples via ELISA to be 47.57 ± 1.21 mg/mL. If this IgG concentration is multiplied by 1.8 (as described by Dunn et al. (2019)), it would yield a predicted RID concentration of approximately 86.6 mg/ml. If colostrum IgG values measured via ELISA by Rudovsky et al. (2008) and Romero et al. (2013) are multiplied by 1.8, it would yield 88.38 mg/ml and 50.81 mg/ml. All of these values are more similar to the average RID concentration measured in the current study and support the hypothesis that methodology differences may affect measured colostrum concentration. Based on these differences, it is suggested that a

different parameter for colostrum quality must be established depending on the method of measurement. Therefore, direct comparison of ELISA and RID values is not recommended. It may be necessary to establish a cutoff value for each specific method of colostrum quality analysis. Otherwise, neonates may be misdiagnosed with failure of passive transfer.

Levieux (2002) found the average colostrum IgG concentration measured by RID to be 47.9±25.5 mg/mL in goats, which is still substantially lower the measured RID value and does not support this hypothesis of methodology difference. Although methodology differences may contribute to a high RID value in our study, there may be other contributing factors, including nutritional differences.

3.5.2. RID Values-Breed Differences

Breed differences can also affect IgG colostrum concentration. Samples in the current study were obtained from a variety of dairy goat breeds including Toggenburg, Saanen, Alpine, and Lamancha. Kessler and colleagues (2019) examined variation of colostrum composition between goat breeds; colostral IgG concentrations varied between 4.8 and 75.0 mg/ml. Colostrum IgG concentration was measured via ELISA. The highest mean IgG concentration was found in the meat-type goats, Boer, (61.0 mg/ml). The lowest mean IgG concentration was found in milk-type goats, Bunte Deutsch Edelziege, (17.9 mg/ml). These data support the hypothesis that meat-type goats have higher colostral IgG concentration compared to milk-type breeds (Kessler et al., 2019). This is similar to the differences observed between beef and dairy cattle colostrum (Guy et al., 1994). Considering these previous findings and the dairy breeds in the current study, one may expect the IgG concentration to be lower than was measured. Studies by Rudovsky et al. (2008), Romero et al. (2013), and Levieux (2002) all had lower

concentrations of IgG, and all studies included dairy goat breeds, Weiße Deutsche Edelziege, Murciano-Granadina, Saanen, and Alpine breeds, respectively. Therefore, the higher value of IgG concentration measured via RID is not suspected to be a result of goat breed differences. The different results obtained may be attributed to physiological characteristics of animals or laboratory differences. It is important to note that where sampling occurred, the dairy was meticulous in feeding and fed high quality dairy alfalfa hay with protein supplementation. This high-quality nutrition may result in high quality colostrum, explaining the high colostral IgG concentrations.

3.5.3. Brix Values

Fresh sample Mean ± standard deviation (median, range) Brix values were 21.2 ± 4.7 (21.7, 9.1-34.0) . Post-thaw Brix determinations were slightly lower at 20.7 ± 4.5 (20.3, 8.7-34.3). There are no available values for goat colostrum Brix comparisons, however these data were similar to what has been observed in dairy cattle. Quigley et al. (2013) reported similar Brix percentage values in dairy cattle of 23.8 ± 3.5 mg/ml.

The association between Brix in fresh and post-thaw samples was highly correlated (r^2=0.94, P<0.0001). The thawed samples were an average of 0.5 mg/ml lower than fresh samples in Brix value, which is consistent with what Argüello et al. (2003) reported in goat colostrum samples. Argüello et al. (2003) reported that repeated freeze-thaw cycles reduced IgG concentration, but not to a significant degree. Similarly, Morrill et al. (2015) showed that in cow colostrum, IgG concentration was unaffected by multiple freeze thaw cycles with the refractometry measurement. Interestingly, Morill et al. (2015) found that when measuring with the RID method, IgG concentration was found to be higher in fresh samples than after multiple

freeze-thaw cycles. It is important to note that even if IgG concentration is unaffected by freezing, overall colostrum quality may still be compromised. Freezing and thawing kills leukocytes, and there is evidence that these cells transfer immune information to the neonate (Van Amburgh, 2018). Therefore, even if the IgG concentration is minimally affected by freezing, there may be additional negative effects that have not been fully studied.

3.5.4. Method Comparison

Overall prediction model relating Brix to RID in goat colostrum was RID (mg/mL)=6.97(Brix)-73.65 (r^2=0.73, P<0.0001). There is no data available for comparison of RID to Brix values in goats, but similar correlations have been reported in cattle. Bielmann et al. (2010) reported a similar correlation of r^2=0.73 (p<.001) between Brix and RID values of colostral IgG in cattle. Dunn et al. (2017) reported a poorer correlation of r^2=0.36. (p=.005) between Brix and RID values of colostral IgG in cattle.

The ELISA method of colostrum quality analysis was not utilized in this study, but Castro (2018) found the relationship between IgG concentration in goats measured by ELISA and clinical refractometer to be r^2=(0.79). Dunn et al. (2017) reported the correlation between cow colostrum IgG concentration measured by ELISA and Brix to be r^2=0.58 (P<.001).

3.5.5. Influencing Factors

Brix determinations on individual samples tended to be influenced by lactation number (P=0.084). In small ruminants, the effect of lactation number on colostral IgG concentration has had varying results in studies. A study completed by Ha et al. (1986) showed that later lactation number goats had a higher colostrum IgG concentration. However, other studies did not observe

a significant effect of lactation number on colostral IgG concentration (Dos Santos et al., 1994; Argüello et al. 2006; Kessler et al. 2019). In cows, a higher lactation number is correlated with increased IgG colostral concentration (Oyeniyi and Hunter, 1978).

Brix determinations on individual samples were not influenced by number of kids per birth. In previous studies, litter size also has unclear effects on IgG concentration in goats. One study reported that colostrum IgG was higher in mothers with twin goats than single goat kids (Csapó et al., 1994). On the other hand, Argüello et al. (2006) and Kessler et al. (2019) did not find a significant difference in IgG concentration dependent on litter size.

No significant differences in Brix or IgG concentration with heat treatment (P=.8127) suggests no degradation in IgG or inadequate heat treatment. This contradicts the findings of Argüello et al. (2003), which found that heating the colostrum to 56 °C for sixty minutes reduces the measurable IgG concentration by 37% when measured with the RID method. However, a bacterial culture on the heat-treated colostrum (in the current study) grew colonies that were TNTC, which suggests that heat treatment was not effective. Further evaluation on farm such as incubator readings is needed to evaluate heat treatment protocol.

3.5.6. Bland-Altman

The Bland-Altman model was used for validation of clinical measurement in comparing the Brix and RID. The Brix variables were converted to predicted IgG concentration with the experimental regression determined in this study and the Bielmann et al. (2010) regression model. The converted values were then compared to RID values using Bland-Altman plots. The mean of 0 in our model (Figure 2) indicates that there is no bias. The limits of agreement (-37.73-37.73) were narrower than the Bielmann model. Overall, this regression model seems to

underestimate high quality colostrum samples and overestimate low values of colostrum concentration. The Bielmann model with the Bland–Altman method showed a fixed bias (predictedRID – experimentalRID) of -36.69 and a wide limit of agreement -86.88 - 13.50. On average the Bielmann model underestimates the amount of IgG and has a poorer level of agreement. A Bland-Altman plot is useful in this study because the correlation between RID and Brix shows the relationship of the variables, how strongly the variable pairs are related, but does evaluate comparability. In this study, 95% of the data points lie with 1.96 standard deviation of the limits of agreement, which is generally considered a validation of the measurements.

3.5.7. Test Characteristics

An appropriate Brix cut-off level is needed to ensure that goat colostrum quality can be assessed on farm with a Brix refractometer, and that high-quality colostrum is fed to kids. For this study, RID values at three different levels (50 mg/ml, 60 mg/ ml, and 79 mg/ml) were evaluated. The RID cut-off of 50 mg/ml was chosen because that is the cut point used in cattle to differentiate between high- and low-quality colostrum (Bielmann et al., 2010). The RID cut off of 79 mg/ml was chosen as this was the calculated weighted average of colostrum concentration fed to kid goats in the current study (data presented in Chapter 4). Finally, 60 mg/ml was chosen as a cut-off point because it is between the goal RID in cattle and the average RID from this study. The optimal Brix cut-off for the RID value of 60 mg/ml was 19. Using this cut point, the sensitivity, specificity, PPV, and NPV were high, indicating that the Brix refractometer can successfully identify colostrum samples with adequate IgG concentration (Table 3). At the RID cut-point of 79 mg/ml, the optimal Brix cut-off was found to be 21, and at the RID cut-point of 50 mg/ml, the optimal Brix cut-off was found to be 18. Sensitivity and specificity were both

high, indicating that the Brix can readily test colostrum above and below the 50 mg/ml and 79 mg/ml cut-off. The diagnostic test characteristics determined in this study for 50 mg/ml, were slightly lower than those determined by Bielmann et al. (2010) in cattle. Bielmann et al. (2010) reported that the optimal cut-off level for a RID value of 50 mg/ml to be 22 for cattle compared to the 18 in this study with dairy goats.

3.6. Conclusions

These data suggest use of a digital Brix refractometer is useful in predicting colostrum IgG concentration for goats similar to what was validated for dairy cattle colostrum. In comparison to reported association in dairy cattle, colostrum containing 50 mg/ml IgG had a Brix value of 18 in goats compared to 22 in cattle (Bielmann et al., 2010). Brix measures were not different between fresh and post-thaw samples and could evaluate pooled colostrum samples, though not as accurately as individual samples. No differences in Brix or IgG concentration with heat treatment suggests no degradation in IgG or inadequate heat treatment. Further research to determine desired colostrum IgG concentration for successful passive transfer is needed.

Chapter 4

RELATIONSHIP BETWEEN GOAT COLOSTRUM IGG INTAKE AND KID GOAT BLOOD IGG CONCENTRATION

4.1. Abstract

Effectiveness of passive transfer is based on delivering sufficient quantity of colostrum in a timely manner relative to birth to achieve adequate blood immunoglobulin G (IgG) concentration that would be protective from disease. There is not sufficient research available on passive transfer in kid goats. The objective of this study was to document serum IgG concentrations in goat kids based on total IgG consumed and determine relationship between total protein and serum IgG concentration. Herd records provided information on amount and timing of colostrum feeding. Blood samples were then collected between 1- and 4-days following birth from kids and serum harvested and frozen for analysis. Total protein (g/dL) and total serum IgG (IgG_1+IgG_2) concentration was measured. Mean ± standard deviation (median, range) serum total protein (n=30) and total IgG concentration (n=57) were 6.0 ± 0.8 g/dL (6.0, 4.5-7.2) and 15.8 ± 7.3 mg/ml (15.3, 3.1-36.1), respectively. Calculated grams of IgG delivered prior to bleeding was 35.0 ± 11.2 g (range: 11.9-64.3). Serum total protein measured between 1 and 4 days of life was highly associated (Figure 4; r_2=0.73; P<0.0001) with serum IgG concentration. Total IgG consumed showed the greatest influence (P=0.0020). Calculated efficiency of IgG absorption was 16.8 ± 8.8% (median 16.4; range 3.6-52.9). Although no assessment of health was performed, these data suggest feeding 35 g IgG can achieve a serum IgG concentration of 15 mg/ml with an associated total protein concentration of 6.0 g/dL. Further research to assess the relationship between health status and IgG transfer to better define passive transfer guidelines.

4.2. Introduction

Colostrum is the unique mammary secretion in all mammals defined as the first milk produced just prior to parturition. At birth, ruminants are agammaglobulinemic due to the inability of uterine and fetal tissues to transfer maternal immunoglobulin molecules (Halliday and Williams, 1979). Most farm animal species, including goat kids, rely on intestinal absorption of colostral immunoglobulins to provide passive humoral immunity, and stimulation of other aspects of immune responses initiated by maternal lymphocytes and other immune regulators (Van Amburgh, 2018).

Feeding of high-quality colostrum is the single most important event in life of a newborn ruminant to ensure viability and survivability. Colostrum quality is based on the concentration of IgG. Numerous studies have documented the importance of colostrum and successful passive transfer of immunoglobulins for health, growth, and survival of animals. In calves, there are long-term benefits documented with adequate colostrum intake such as improved rate of gain and feed efficiency (Robison et al., 1988), reduced age at first calving, and improved milk production (DeNise et al.,1989). In goats, similar long-term benefits have been documented showing increased rate of gain in relation to elevated serum IgG concentrations 24 hours after parturition (Massimini et al., 2007).

Passive transfer is a form of acquired immunity that is characterized by intact immunoglobulin absorption across the small intestine from colostrum directly after birth. Passive immunity in the neonate relies on the ability of intestinal cells to transport macromolecules from gut lumen into the bloodstream. Immediately after birth, intestinal uptake is nonselective and transient in cattle, sheep, goats, and swine (Hurley and Theil, 2011). This species-specific unique uptake is controlled by nutrient independent factors present in colostrum. The ability of a farm-

animal to absorb immunoglobulins decreases a day or two after birth until the intestine no longer absorbs macromolecules in a process deemed intestinal closure (Pond et al., 2011). In goat kids, peak IgG concentration was observed 24 hours after birth (Argüello et al., 2004).

Failure of passive transfer occurs when the threshold concentration of IgG for a specific species is not reached before closure occurs, either via inadequate colostrum quantity or quality within the first hours of life. The threshold concentration of IgG is the minimum amount of IgG necessary in sera to ensure the vitality of the neonate, which is determined experimentally for each individual species. Failure of passive transfer is the primary cause of increased susceptibility to infectious disease, increased morbidity, and increased mortality rates in newborn kids (O'Brien et al, 1993). Therefore, high-quality colostrum is essential for kid health.

Passive transfer in kid goats is measured through serum immunoglobulin levels. In one study (O' Brien and Sherman, 1993), kids were fed their individual dam's colostrum and the mean serum IgG level varied from kid to kid. The mean serum immunoglobulin level for all kids was 11.70 mg/ml. Overall, this study showed a significantly higher mean serum IgG level in kids that survived to weaning versus those that died and confirmed that kid health and survival is associated with passive transfer of immunoglobulins. From this study, a recommended minimum of 12.00 mg/ml of serum IgG was required in newborn kids for vitality. In calves, a recommended minimum of 10.00 mg/ml of serum IgG is required for calf vitality (Elizondo-Salazar and Heinrichs, 2009).

Passive transfer is affected by volume of colostrum ingested, colostral IgG concentration, and total amount of immunoglobulin fed (Stott and Fellah, 1983). In calves, Stott and Fellah (1983) found that IgG colostrum concentration is more important than the quantity of the colostrum consumed if total consumed IgG was the same. Calves that consumed the higher

concentration of colostrum at a lower volume had higher levels of IgG in the blood. In goat kids, Argüello et al. (2004) showed that quantity of IgG consumed had a positive high correlation with IgG blood concentrations within the first 72 hours of life. Michanek and Ventorp (1989) explored the effect of age at first feeding on passive transfer. They found that calves fed colostrum at one hour old absorbed more colostrum in later feedings compared to other first feeding times

With the documented importance of adequate passive transfer, production managers must have a method to evaluate the effectiveness of passive transfer. Enzyme-linked immunosorbent assay (ELISA) and total protein measurements of serum are two methods to evaluate passive transfer.

The ELISA method can be used to measure immunoglobulins in colostrum and involves antibody-antigen interactions. The IgG concentration of goat serum samples were measured via ELISA by Rodríguez et al. (2009). The IgG concentration of cow plasma was measured via ELISA by (Gelsinger et al., 2015).

Measuring total protein of serum can be used to evaluate passive transfer of IgG. Serum total protein from neonates can be evaluated via a refractometer tool. Studies have shown refractometry serum total protein concentration is closely correlated with serum IgG concentration in lambs (Massimini et al., 2006). Therefore, it has been suggested as a valuable method to measure passive transfer status. In calves, a serum total protein level of 5.0 g/dL predicts a serum IgG level of 10 mg/ml, which is indicative of successful passive transfer (Swan et al., 2007). Another study found that a serum protein concentration of 5.2 g/dL was equivalent to 10 mg/mL serum IgG_1 in calves (Tyler et al., 1996). There is minimal data available regarding total protein and passive transfer in goats.

Although the correlation between serum IgG and kid health has been documented, there are varying results regarding colostrum consumed and serum IgG concentration. In one study, Dos Santos et al. (1994) did not find a relationship between consumed IgG concentration and serum IgG concentration in one day old kids. In calves, however, it has been observed that those fed a higher concentrated colostrum will have greater IgG absorption (Castro et al., 2005). Constant et al. (1994) showed the effect of colostrum concentration and volume on serum IgG levels in kid goats.

In dairy cattle, there is extensive research on passive transfer analysis (Beam et al., 2009), but this information is not available for goat kids. The overall objective of this study was to document serum IgG concentrations in goat kids based on total IgG consumed and determine the relationship between total protein and serum IgG concentration.

4.3. Materials and Methods

All procedures were reviewed and approved by the Pennsylvania State University Institutional Animal Care and Use Committee (IACUC#: 47927).

4.3.1. Housing, Animals, and Feeding

A commercial, Humane-Certified, 900-doe goat dairy located in Humboldt County, California was the source for sample collection during 2018. Local veterinary service is used for routine health care and a consulting veterinarian provides consultation on herd health management, reproductive assessment and nutritional formulation. Goats are housed in three large greenhouse barns with four group pens capable of loosely housing 100-150 goats per pen. Most pens were maintained at 65-85% of capacity as the dairy was continuing to increase herd

size. The dairy used extended lactations and out-of-season breeding to maintain a milk supply to the associated processing plant year-round. Loose housing areas were bedded with locally sourced almond shells.

Animal grouping was based on production stage and varied with percent of herd in lactation. Production groups included extended lactation (>300 days in milk), early lactation (<40 DIM), and high production (>40 DIM). Dry does were divided into a 2-group system of far-off (< 30 days prior to kidding) and close-up (>30 days prior to kidding) does. Pregnant doelings were placed into the close-up pen at least 60 days prior to kidding. After kidding does were placed in a post-partum pen for a few days before being moved into the early lactation group. Kids were fed colostrum and moved to a separate kid barn and fed milk replacer and starter grain.

Dairy goat breeds represented on the farm included Toggenburg, Saanen, Alpine, and Lamancha. Most does were crossbreeds of two or more of these breeds. Farm management had an electronic herd record system as well as a parlor that provided daily milk weights. All data relative to doe age, kidding date, kid numbers, live vs. dead, birth weights, time from kidding to sampling and lactation number were compiled. Data relative to days dry, previous lactation length and production were also collected.

Lactating pens were fed a total mixed ration (TMR) twice daily. Far-off dry and close-up dry pens were fed the TMR that was top-dressed with additional grains [Table 2: Feed Analysis in Chapter 3].

4.3.2. Colostrum Management

Fresh colostrum samples were collected from does and pooled (3 to 6 does/sample) within a group prior to heat treatment. Colostrum was heated to 135 °F (60 °C) for 1 hour.

Only heat-treated pooled samples were fed to female kids. Individual doe samples were collected as part of the study and this colostrum was only fed to male kids. Colostrum was fed in two doses, 6 to 8 oz per dose (with three exceptions). In one exception, a kid was fed a single large dose of 16oz of colostrum. In two other cases, kids were fed a larger second dose of 10 oz of colostrum. Kids were bottle or tube fed for the first dose of colostrum as soon as possible after birth and kids were bottle, tube, or bucket fed for the second dose of colostrum a couple hours later.

4.3.3. Biological Sampling

Blood samples were collected by a veterinarian from kid goats from the jugular vein between 1- and 4-days of age following birth. A standard operating procedure for proper sampling to minimize potential bacterial contamination was developed and provided to all farm personnel involved in sample collection. Samples were centrifuged initially in the field before processing at the local veterinarian. Samples were collected over a period between March 30 and April 13, 2018. Samples were collected into 1.8 mL tubes with a cap and labeled with a kid identification number and collection date, frozen (-20°C) and shipped on ice to Pennsylvania State University.

4.3.3.1. Serum Measurements

Serum was evaluated for total protein determination using a digital refractometer (Palm Abby, MISCO, Solon, OH) and total IgG concentration quantified via ELISA using a commercial goat-based assay kit (ELISA, ZeptoMetrix, Buffalo, NY). Prior to testing, serum samples were allowed to reach room temperature.

4.3.3.2. Refractometer

Total protein determination (g/dL) was determined by digital refractometer (MISCO, Solon, OH). After reaching room temperature, the serum sample was inverted at least twice. The refractometer was calibrated with a drop of distilled water. A drop of serum was transferred with a pipette onto the prism of the refractometer. The sample cover was lowered, and the sample read on "Total Protein" mode. The refractometer was washed with distilled water and wiped with a clean paper towel between readings. This measurement was completed in triplicate for each sample.

4.3.3.3. ELISA

Total serum IgG (IgG_1+IgG_2) concentration was determined by goat IgG ELISA (ELISA, ZeptoMetrix, Buffalo, NY). The ELISA method was preformed according to the manufacturer's instructions for Goat IgG ELISA Kits produced by Zeptometrix. The serum and ELISA kit were allowed to reach to room temperature by sitting on the bench top. The serum was diluted to 1:300,000 with a threefold 1:70 dilution. Each sample was run in triplicate. The standard dilution series were run in duplicate for each assay. Vortexing, wetting pipette tips, and changing gloves

were used to reduce dilution errors. Samples were measured with a mircoplate reader at an absorbance of 450 nm. Samples with a coefficient of variation greater than 10 were repeated.

4.3.3.4. Colostrum Measurements

Colostrum immunoglobulin content was evaluated with a Brix refractometer (Palm Abby, MISCO, Solon, OH) and quantified by RID using a commercial goat-based assay kit (Triple-J Farms, Bellingham, WA).

On farm digital Brix determinations were performed in duplicate on the fresh individual doe and pooled colostrum samples. Pooled samples were also evaluated following heat treatment. A subsample of fresh and pooled (before and after heat treatment) colostrum was frozen and shipped to Penn State University for a post-thaw Brix determinations and measurement of total IgG concentration using RID (Triple-J Farms, Bellingham, WA). Before measurements, colostrum samples were allowed to reach room temperature by either using a hot water bath set to 50° C or by sitting on the bench top.

4.3.4. Statistical Analysis

Serum total protein and ELISA data were tested for normality using Proc UNIVARIATE in SAS (ver. 9.4, Carey, NC). Both total protein and ELISA determinations of serum content were analyzed by ANOVA (Proc GLM) to determine the influence of total IgG consumed by kid, volume of colostrum consumed, concentration of colostrum consumed, time of colostrum consumption, and age of blood draw. Relationship between colostrum consumed (total IgG, IgG concentration, volume) determined by Brix and RID to serum total protein and IgG concentration was established using correlation analysis (Proc CORR) and further explored with ANOVA

where the model included various sample parameters as covariants. Relationship between serum total protein and IgG concentration determined by ELISA was established using correlation analysis (Proc CORR). A predictive relationship between total protein and IgG concentration was determined using regression modeling (Proc REG).

4.4. Results

4.4.1. Averages and Correlation Coefficients

Hemolyzed serum samples (determined subjectively by evaluating color of serum) resulted in extremely high total protein and were removed from data analysis. Mean ± standard deviation (median, range) serum total protein (n=30) and total IgG concentration (n=57) were 6.0 ± 0.8 g/dL (6.0, 4.5-7.2) and 15.8 ± 7.3 mg/ml (15.3, 3.1-36.1), respectively. Calculated grams of IgG delivered at first feeding and total prior to bleeding was 17.6 ± 6.3 g (range: 6.0-39) and 35.0 ± 11.2 g (range: 11.9-64.3), respectively. Time to first feeding averaged 106 ± 136 mins (median: 60 min). Serum total protein measured between 1 and 4 days of life was highly associated (Figure 4; r_2=0.73; P<0.0001) with serum IgG concentration.

Figure 4. Total Protein vs. IgG Concentration of Serum

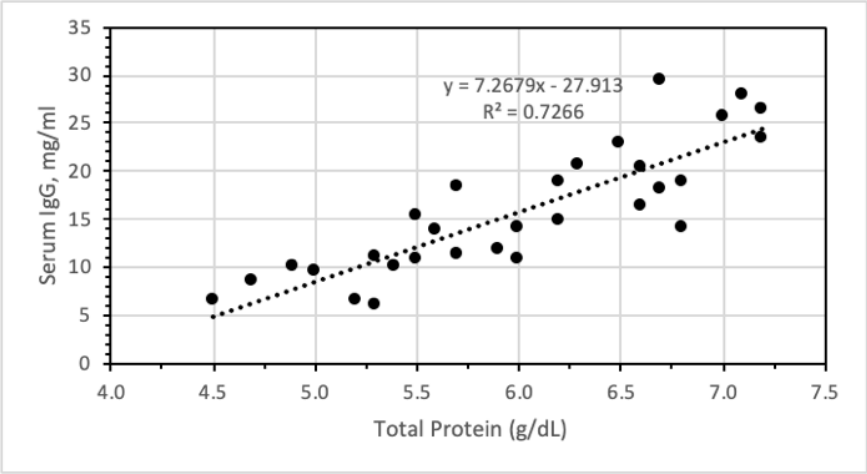

Figure 4. Relationship between serum IgG concentration and total protein concentration in goat kids ranging from 1-4 days of age.

4.4.2. Influencing Factors

Incorporation of age at bleeding (P=0.08) only slightly improved the relationship between total protein and serum IgG concentration (r^2=0.76, P<0.0001). Total IgG consumed showed the greatest influence (P=0.0020) with no effect of method of feeding or time to feeding. Amount of IgG fed at 1st feeding (P=0.0069), volume at first feeding (P=0.10), time to second feeding (P=0.061) and age (P=0.047) accounted for more variation (r^2=0.45, P=0.0093) in serum IgG concentration than total IgG consumed (r^2=0.36, P=0.013). Mean total consumed IgG amount was 35.0 ± 11.2 g (median 36.1; range 11.9-64.3). Weighted mean of consumed IgG concentration was 79.80 mg/ml.

4.4.3. Efficiency of Absorption

Assuming blood volume at 10% of birth weight, total blood IgG mass was determined from measured serum IgG concentration using the equation: [serum IgG (mg/mL at d 1) × serum volume (mL)/IgG intake (mg)] × 100. Calculated efficiency of IgG absorption was 16.8 ± 8.8% (median 16.4; range 3.6-52.9). Total IgG consumed was negatively associated (P=0.012) with IgG absorption efficiency. Time to second feeding (P=0.023) and IgG amount at second feeding (P=0.015) explained the greatest amount of variation (r_2 = 0.23, P=0.0016) in calculated efficiency of colostrum absorption.

4.5. Discussion

4.5.1. Total Protein

Mean ± standard deviation (median, range) serum total protein (n=30) of the kid goats were 6.0 ± 0.8 g/dL (6.0, 4.5-7.2) which is similar to data reported by Batmaz et al. (2019), who found that total protein (g/dl) was 6.00 ± 0.15 in 1-day-old kid goats.

4.5.2. Serum IgG Concentration

Total serum IgG concentration (n=57) was 15.8 ± 7.3 mg/ml (15.3, 3.1-36.1), which is higher than the recommended minimum of 12.00 mg/ml of serum IgG that is required in newborn kids for vitality (O' Brien and Sherman 1993). Successful passive transfer can also be measured as more than 3.0 g IgG/kg BW in serum (Constant et al., 1994). Castro et al. (2007) determined 4 g IgG/kg BW in serum within 24 h provided adequate passive transfer [according to O'Brien and Sherman's (1993) definition] for 80% of the kids studied. Using the criteria of O'Brien and Sherman, 66% of kids in the current study would be considered to have sufficient

passive transfer and 34% experienced failure of passive transfer. Additionally, the mean total serum IgG concentration in our study is higher than reported values in previous studies. O'Brien and Sherman (1993) found the mean serum IgG level for kids to be 11.70 mg/ml. Batmaz et al. (2019) reported a mean serum IgG level for kids to be 11.32 mg/ ml at 3 days old.

Serum total protein measured between 1 and 4 days of life was highly associated with serum IgG concentration (Figure 1; r_2=0.73; P<0.0001). Batmaz et al. (2019) also found a positive correlation between total protein and IgG on 1-day-old kid goats (r_2=0.19, P<0.001), however the correlation coefficient was lower. Massimini et al. (2006) also found a highly correlated relationship between serum IgG concentration in 1-day-old lambs and refractometry serum total protein (P < 0.001; r_2 = 0.848). Overall, this data suggests that total protein of serum is an effective method to evaluate the amount of IgG in serum without the elaborate and time intensive ELISA or RID test.

4.5.3. Influencing Factors

Total IgG consumed showed the greatest influence on serum IgG concentration (P=0.0020), which is consistent to other studies which showed that IgG consumed is the greatest single factor that influences IgG concentration. This is similar to a study by Argüello et al. (2004) who reported that quantity of IgG consumed had a positive high correlation with IgG blood concentrations within the first 72 hours of life. Amount of IgG fed at 1st feeding (P=0.0069), volume at first feeding (P=0.10), time to second feeding (P=0.061) and age (P=0.047) accounted for more variation (r_2=0.45, P=0.0093) in serum IgG concentration than total IgG consumed (r_2=0.36, P=0.013). In one study, Dos Santos et al. (1994) did not find a relationship between consumed IgG concentration and serum IgG concentration in 1-day old

kids. In calves, however, it has been observed that those fed a higher concentrated colostrum will have greater IgG blood concentration (Castro et al., 2005). Constant et al. (1994) showed the effect of colostrum concentration and volume on serum IgG levels in kid goats. The kids fed a higher dose colostrum (3.0g of IgG/kg) had a higher serum IgG concentration of 17.27 ± 2.44 mg/ml compared to those fed a lower dose of colostrum (1.5 IgG/kg) with a concentration of 14.60 ± 2.88 mg/ml. Michanek and Ventorp (1989) found that calves fed colostrum at one hour old absorbed more colostrum in later feedings compared to other first feeding times.

4.5.4. Efficiency of Absorption

Calculated efficiency of IgG absorption was 16.8 ± 8.8% (median 16.4; range 3.6-52.9) in kids fed a weighted average of colostrum with a concentration of 79.80 mg/ml. This is a lower efficiency of absorption than expected from previous studies. Rodriguez et al. (2009) calculated the apparent efficiency of absorption in kid goats to be 13.3, 13.3, 14.9, and 24.4% in groups of IgG concentration of 20 mg/ml, 40 mg/ml, 60 mg/ml, and 80 mg/ml respectively. The AEA was 24.4% in the 80 mg/ml group in Rodriguez's study, however, at a similar concentration of 79.80 mg/ml in our study the AEA was lower at 16.8%. Previous studies have reported that increasing the immunoglobulin concentration in colostrum improved immunoglobulin absorption at the same amount of total immunoglobulin fed, so the high colostral concentration in our study led to a relatively high AEA, even if it was lower than expected. Johnson et. al. (2007) calculated AEA in calves to be around 25%, which is similar to that of kids fed at the highest IgG concentration in Rodriguez's study, and higher than the AEA measured in our study.

Calculated total grams of IgG consumed prior to bleeding was 35.0 ± 11.2 g (range: 11.9-64.3), and these data suggest feeding 35 g IgG can achieve a serum IgG concentration of 15

mg/ml with an associated total protein concentration of 6.0 g/dL. Research with calves indicates reduced disease prevalence and severity with serum IgG concentrations ≥ 15 mg/ml (Furman-Fratczak et al., 2011). Based on this calf work, it would seem desirable to attempt to achieve a serum IgG concentration of 15 mg/ml in goat kids, which is higher than previous thresholds of successful passive transfer identified for goats in previous studies.

4.6. Conclusions

These data suggest that total protein of serum is an effective method to evaluate the amount of IgG in serum without the elaborate and time intensive ELISA or RID test. Although no assessment of health was preformed, these data suggest feeding 35 g IgG can achieve a serum IgG concentration of 15 mg/ml with an associated total protein concentration of 6.0 g/dL. Further research to assess the relationship between health status and IgG transfer to better define passive transfer guidelines.

Chapter 5

CONCLUSIONS

This study evaluated colostrum quality in goats and determined the relationship between Brix reading and immunoglobulin G (IgG) concentration. Additionally, serum IgG concentrations were documented in goat kids based on total IgG consumed and the relationship between total protein and serum IgG concentration was evaluated. The Brix refractometer was determined to be a useful method in predicting colostrum IgG concentration for goats, similar to what has validated for dairy cattle colostrum. In comparison to reported association in dairy cattle, colostrum containing 50 mg/ml IgG (measured via radial immunodiffusion (RID)) had a Brix value of 18 in goats compared to 22 in cattle (Bielmann et al., 2010). Further data, such as enzyme-linked immunosorbent assay (ELISA) values of colostrum, would further validate these findings. Brix measures were not significantly different between fresh and post-thaw colostrum samples and could be used to evaluate pooled colostrum samples, though not as accurately as individual samples. No differences in Brix or IgG concentration with heat treatment suggests no degradation in IgG or inadequate heat treatment in this current study. Total protein was found as an effective method to evaluate the amount of IgG in serum without the elaborate and time intensive ELISA or RID test. Although no assessment of health was preformed, these data suggest feeding 35 g IgG can achieve a serum IgG concentration of 15 mg/mL with an associated total protein concentration of 6.0 g/dL. This is a higher value than the published criteria of 12 mg/mL serum IgG concentration for successful passive transfer in kid goats (O'Brien and Sherman, 1993). Further research is needed to determine desired colostrum IgG concentration for successful passive transfer, to further evaluate the factors that influence absorption efficiency, and to assess the relationship between health status and IgG transfer. Further data including long

term health data on goat kids in comparison to serum IgG concentration would be useful in evaluating a threshold for passive transfer.

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ACADEMIC VITA

Elizabeth Vyvyan St. Clair

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EDUCATION

The Pennsylvania State University — **University Park, PA**
Schreyer Honors College — *Graduation: May 2020*
Bachelor of Science in Veterinary and Biomedical Sciences

PROFESSIONAL EXPERIENCE

Laboratory Assistant, Central Milk Testing Lab — **University Park, PA**, *January 2019 – May 2020*
- Led an independent research project on evaluating colostrum quality and passive transfer in kid goats with intent to publish scientific papers in the upcoming months
- Developed and wrote protocol for Enzyme-Linked Immunosorbent Assay (ELISA)
- Collected blood, metritis, and milk samples from dairy cows for field research
- Learned to: prepare culture media for bacteriology, autoclave laboratory equipment, and maintain biosecurity of the lab

Dairy Intern, Kreider Farms — **Manheim, PA**, *Summer 2018*
- Worked over 500 hours on an 1800 cow milking dairy in various positions
- Included experience with microbiology of animal samples, sick cow treatment, artificial insemination training, and overall large herd management practices

Education Aide, ZooAmerica — **Hershey, PA**, *May 2017-May 2020*
- Prepared diets, administered medication, maintained cleanliness, and introduced enrichment to the education animals at the zoo
- Performed educational animal programs at the zoo and local schools
- Learned the importance of record-keeping and data entry in animal care

Volunteer Puppy Raiser, Susquehanna Service Dogs — **Grantville, PA**, *2019-May 2020*
- Raised a service dog (SSD Avalon) for 4 months in my home and currently sit other SSD dogs
- Facilitated in at least 2 hours daily of active in-home training with service dogs, attended weekly training classes, and brought dogs to public places for experience such as grocery stores, libraries, restaurants, airports, etc.

EXTRACURRICULARS

Pre-Vet Club — **University Park, PA**, *Fall 2016 – May 2020*
- Held an elected officer role every semester as a student including President, Secretary, Agricultural Student Council Chair, and Pre-Vet Symposium Chair
- Planned the American Pre-Veterinary Symposium, a national symposium educational event that spanned three days and included 600 students from across the world

Reproduction Research Team — **University Park, PA**, *Fall 2016 – May 2020*
- Observed and recorded signs of estrus in dairy cattle for use in corpus luteum research

HONORS

Evan Pugh Scholar Award — *Spring 2019*
- Prestigious award is given to students in the top 0.5% of the Penn State class

Coaly Society — *Spring 2018-May 2020*
- Member of highly selective honorary society for College of Agricultural Sciences students who have demonstrated leadership excellence and satisfactory academic performance

APVMA Scholarship — *March 2019*
- Nationwide scholarship was awarded based on leadership and Pre-Vet Club participation.