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The Effects of Poor Maternal Nutrition During Gestation on Colostrum and Milk Quality and Immunoglobulin G Concentrations in Sheep

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Abstract

Maternal over- and under-nutrition have negative effects on the growth and development of offspring. Colostrum and milk are critical to neonatal development, and composition and quality of colostrum and milk may be influenced by maternal factors, including diet. In this study, 46 pregnant ewes received one of three diets, 60% (RES), 100% (CON), or 140% (OVER) of National Research Council (NRC) nutrition requirements for total digestible nutrients from d 30 of gestation until parturition. Colostrum samples were collected within 24 hours of parturition. Milk samples were collected on d 3 and d 21 postpartum. Total solid concentrations of samples were measured utilizing Brix refractometry. Total solids decreased 7.9% at d 3 and 8.6% at d 21 compared with d 0 (d 0: 1.15 ± 0.013, d 3: 1.06 ± 0.002, d 21: 1.05 ± 0.001; $P < 0.0001$). There were no detectable effects of maternal diet or interaction of maternal diet and time point on total solids ($P > 0.35$). Colostral IgG concentrations were significantly impacted by treatment group (RES: 98.99 g/L ± 15.76 g/L, CON: 154.05 g/L ± 21.08 g/L, OVER: 173.14 g/L ± 12.30 g/L; $P < 0.0001$). Further analyses of milk components and lamb serum to evaluate the success of passive transfer in offspring from ewes fed a poor diet during gestation are warranted.

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Introduction

As the global population continues to rise, the demand for sustainable and efficient food production is growing. The growth and health of livestock plays a vital role in fulfilling this demand, and in contributing to food security and the global economy. In the food animal production industry, livestock may be restricted-fed due to decreased availability and quality of feed due to seasonal variation, harsh climates, insufficient food production, and improper management practices (Hoffman et al., 2017). Production animals may be over-fed due to a variety of management practices, including flushing, ad-libitum feeding, and grazing of rich pastures (Hoffman et al., 2017). Both restricted and excess diets during gestation result in poor maternal nutrition. Poor maternal nutrition is defined as excess or lack of overall nutrients or specific nutrient classes, such as protein or minerals, in the diet of the dam during gestation (Hoffman et al., 2017; Reed et al., 2014). Poor maternal nutrition results in immediate and long-term phenotypic alterations in offspring (Reed et al., 2014). Although studies have evaluated the effects of restricted and over-feeding during lactation, little scientific data have been produced on the effects on lactation after the consumption of a poor maternal diet during gestation. Quality colostrum is vital to the survival of neonatal lambs, in order to provide essential nutrients for growth, including immunoglobulin G (IgG). Several studies have previously been conducted at the University of Connecticut evaluating the negative effects of poor maternal diet during gestation on growth and development of offspring (Hoffman et al., 2016), but no lactation data have been previously evaluated in this sector, thus warranting further study and investigation.

Effects of Poor Maternal Nutrition on Gestation and Neonatal Development

The current literature focuses on the effects of poor maternal nutrition during gestation on changes in gene and protein expression and stem cell function in bone, adipose, and muscle, due to the importance of those tissues in the livestock industry (Hoffman et al., 2017). Poor maternal nutrition during gestation contributes to altered offspring muscle growth during early fetal development that continues to persist throughout the fetal development stage (Gauvin et al., 2020), including effects on reducing muscle fiber number and altering regulators of muscle growth (Reed et al., 2014). In lambs from restricted and over-fed ewes, muscle fiber cross-sectional area decreased 15% and 17% and muscle lipid content increased 92.5% and 212.4% respectively, when compared with control-fed ewes (Reed et al., 2014), warranting further investigation on the generational effects of poor maternal nutrition during gestation.

The restriction or excess of nutrients during gestation both negatively impact offspring, including alterations in body size, concentrations of circulating growth factors, and endogenous metabolic hormone concentrations in offspring postnatally (Hoffman et al., 2017). Poor maternal nutrition may cause drastic consequences in the quality of offspring, including reduced offspring muscle mass, altered muscle composition, increased adipose tissue in carcasses, disruption of metabolic regulation in offspring, and negative impacts on organ development (Hoffman et al., 2017). Poor maternal nutrition may also result in significant impacts on postnatal muscle gene expression and postnatal phosphorylation of Akt in offspring at three months of age (Reed et al., 2014).

Swanson et al. (2008) determined that nutritional modification during the final two-thirds of pregnancy greatly impacted the birth weight of lambs, mammary gland development, and

colostrum components. On day 50 of gestation, ewes were assigned randomly to one of three nutritional diets, 60% (RES), 100% (CON), or 140% (HIGH) of National Research Council (NRC) requirements. Gestation length was reduced in HIGH ewes when compared to CON and RES ewes. Reduced lamb birth weight was noted in HIGH ewes that lambed earlier, and in RES ewes that had gestation lengths similar to that of CON ewes.

Hammer et al. (2011) conducted two experiments investigating the influence of maternal selenium supply and plane of nutrition on health of neonatal lambs. On days 40 and 50 of gestation, for the first and second experiments, respectively, ewes were assigned randomly to one of three nutritional diets, 60% (RES), 100% (CON), or 140% (HI) of NRC requirements. Similar to the results of Swanson et al. (2008), gestation length was decreased in ewes on the HI nutritional plane, and birth weight was decreased in lambs from RES and HI dams when compared to lambs from CON dams. Postnatal effects of poor maternal nutrition impact a variety of tissues and organ systems in offspring, including the development of the immune system in these neonates.

The Synepitheliochorial Placenta in Sheep

Ruminants, including sheep, have synepitheliochorial placentas, in which the placenta acts as barrier, preventing antibody transfer to the fetus during gestation, making the ingestion and absorption of colostrum extremely important for immune transfer immediately following birth (Borghesi et al., 2014). The six tissue layers in the synepitheliochorial placenta that act as a physical boundary between maternal and fetal circulation are the maternal capillary endothelium, maternal connective tissue, maternal epithelium, trophoblasts, embryonic connective tissue, and fetal endothelium (Borghesi et al., 2014). As the ovine placenta prevents maternal transfer of immunoglobulins (Ig) to the fetus during gestation, lambs rely heavily on colostrum for

successful passive transfer of IgG from the dam. The five different classes of immunoglobulins, or antibodies, are IgA, IgD, IgE, IgG, and IgM, with IgG being the most prominent in sheep colostrum (Borghesi et al., 2014). One of the most important variables in immune system development is the Ig concentration of plasma, particularly IgM and IgG (Hernandez-Castellano et al., 2015). Lambs, like all progeny of ruminant species, are born hypo-gammaglobulinemic (Hernandez-Castellano et al., 2015). Due to the structure of the layers of the synepitheliochorial placenta, the immune system of neonatal ruminants is acquired passively through colostrum (Borghesi et al., 2014). Colostral IgG concentrations become drastically reduced within 24 hours of parturition (Borghesi et al., 2014). Thus, immediate colostrum ingestion is necessary in order for the neonate to acquire rapidly diminishing IgG present in the secretion.

IgG holds vital importance in the successful development of ovine offspring. Immunoglobulins are absorbed via pinocytosis, primarily in the jejunum and ileum of the small intestine, and are then transported into lymphatic tissues, enter the circulatory system through the thoracic duct, and provide essential immunity to the neonate (Borghesi et al., 2014). Three critical periods related to immune system development in lambs include colostrum feeding, milk feeding, and weaning (Hernandez-Castellano et al., 2015). Regardless of the colostrum source, as there is similar IgG homology in bovine and ovine species (al-Sabbagh et al., 1995), or timing of the first colostrum feeding, there is a distinct increase in IgG concentrations in lamb serum during the first 24 hours after birth, with a maximum IgG concentration 24 to 48 hours after birth (Hernandez-Castellano et al., 2015). Adequate passive immune transfer occurs in lambs that have ingested at least 30 g of IgG (Alves et al., 2015). While lamb IgG plasma concentrations increase exponentially, Ig concentrations diminish during lactogenesis rapidly. Linear regression

analysis projects IgG concentrations to diminish to 0 mg/mL by 23 hours postpartum in sheep (al-Sabbagh et al., 1995).

Colostrum Composition

Colostrum, the first milk produced in the beginning stages of lactogenesis in the first few days postpartum, is of extreme importance due to its immunological components passed passively to the offspring (Ballard & Morrow, 2013). In sheep, colostrum is rich in vital nutrients, including high IgG concentrations, with a progressive decline noted over six milkings, until mature milk nutrient concentrations were observed (Alves et al., 2015). In one study, the concentration of IgG sharply declined from 25.36 mg/mL at 6 hours postpartum to 11.73 mg/mL at 24 hours postpartum, with further follow up times resulting in values close to 0 mg/mL (Alves et al., 2015).

Kessler et al. (2019) reports findings of IgG concentrations and composition of colostrum samples obtained between 10 and 390 minutes after parturition from 100 ewes of varying breeds. The results of this study indicated that fat, protein, and lactose constituents varied widely across breeds, with fat and protein compositions ranging from 1.1 to 24.8% and 7.3 to 30.5%, respectively. Age of the dam did not have a significant effect on fat and protein composition in this study. Fat and protein concentrations were found to be significantly higher in ewes of meat-type breeds, as well as in ewes bearing multiple lambs. A large range of colostral IgG concentrations was noted across ovine breeds, ranging from 6.2 to 65.4 mg/mL. Colostral IgG concentrations from some RES-fed Rambouillet ewes have even reached 127.7 mg/mL (Swanson et al., 2008).

Significant variation in colostral IgG concentration exists between and within breeds, and may be affected by other maternal factors, such as age and parity (Tabatabaei et al., 2013;

al-Sabbagh et al., 1995). IgG is transported by the neonatal Fc receptor (Borghesi et al., 2014), and inter-breed variation in colostral IgG concentrations are potentially associated with polymorphisms in the neonatal Fc receptor gene (Tabatabaei et al., 2013). In addition, colostrum quality and quantity appear to be directly related to, and regulated by, maternal nutrition (Swanson et al., 2008). Present data suggest that maternal diet during gestation impacts neonatal health and the ability of the neonate to acquire passive immunity of immunoglobulins via colostrum (Hammer et al., 2011).

Effects of Poor Maternal Nutrition on Lactation

Maternal diet contributes to the body composition of both the offspring and the dam, and can impact lactation.. Body condition score (BCS) is a quantitative and subjective measure of body fat and muscling of animals on a scale of 1 to 5, with a 1 indicating a very thin animal and a 5 indicating an obese animal. When ewes fell between a BCS range of 2.5 to 3.5, a normal and acceptable BCS, at the time of lambing, BCS had no significant effect on colostral IgG concentrations (al-Sabbagh et al., 1995). Alves et al. (2015) determined that colostrum production was reduced in primiparous ewes with a BCS less than 2.75 when compared to multiparous ewes of the same BCS. No effect was observed between primiparous and multiparous ewes when BCS was over 2.75. Maternal diet during gestation up until the time of parturition has significant impacts on maternal body and milk composition, yet realimentation of diet during the lactation period may help in negating some of these effects.

In experimental animal models, chronic undernutrition of dams correlates with deleterious effects on milk composition, and reduced nutrient intake of the young, resulting in poor growth (Rasmussen, 1992). When fed RES and HIGH diets, based on NRC requirements, ewes produced colostrum of reduced weight and volume (Swanson et al., 2008). Restricted-fed

ewes have been documented to produce greater IgG concentrations in early lactogenesis. Colostral IgG concentrations were greater in RES fed ewes than in CON and HIGH fed ewes, while total IgG production was reduced in RES and HIGH fed ewes when compared to CON fed ewes due to the reduced volume of colostrum produced (Swanson et al., 2008). Poor maternal nutrition was found to increase colostrum density in RES ewes when compared to CON ewes, with HIGH ewes falling intermediately (Swanson et al., 2008). RES and HIGH ewes also had reductions in total butterfat, protein, lactose, and solids not fat components on a per gram basis when compared to CON colostrum (Swanson et al., 2008). Ad libitum feeding during lactation may aid in reversing the effects of maternal food restriction during gestation, or realimenting the animal. In a study restricting feed intake to 40, 60, or 80% ad libitum in lactating rats, an average reduction in milk production was observed in restricted animals when compared with animals fed ad libitum, but total lipid and total protein concentrations in the milk were not significantly affected by dietary treatments (Grigor et al., 1987).

Similar to sheep, in humans, maternal nutrition status influences the quality and quantity of milk produced by the mother (Miranda et al., 1983). A striking reduction in colostral IgG concentrations due to malnutrition has been noted, and it is not until 4 weeks postpartum that the difference between malnourished and adequately fed mothers became insignificant (Miranda et al., 1983). A direct correlation between milk output and dietary protein intake has been identified, with concentrations of all milk proteins evaluated, including IgG, C3, C4, lysozyme, and albumin, becoming decreased as lactation progressed (Miranda et al., 1983). Overall, during gestation and lactogenesis across species, maternal nutrition plays a vital role in milk quantity and quality.

Transitional and Mature Milk Composition

Pavic et al. (2002) states that the quantity of milk produced, chemical composition, and physical properties of milk in sheep are influenced by genetics, including breed and genotype, age, parity, body weight, number of lambs, stage and number of lactation, management, and milking methods. Over a 240 day lactation period, with early lactation defined as the first 60 days postpartum, the midpoint stage from day 61 to day 180, and the final stage defined as day 181 to day 240, the stage of lactation was found to have significant influence on the chemical composition of milk. Milk samples were evaluated for total solids, milk fat, crude protein, and lactose anhydrite concentrations, with the stage of lactation having significant influence over all parameters analyzed, and milk fat being most prone to variability between the time points.. In early lactation, milk contained significantly lower contents of total solids, fat, and protein, while protein content was highest at 18.09% during the final stage of lactation. Lactose content was highest at 4.97% during early lactation, and lowest at 4.09% during the final stage.

In humans, the production of transitional milk, approximately 5 days to 2 weeks postpartum, involves a period of greatly increased milk production, while sharing some characteristics of colostrum, with milk being considered fully mature by 4 to 6 weeks postpartum (Ballard & Morrow, 2013). There is a great shift in milk composition in the first month of the neonate's life, while mature milk is rather consistent in composition, with subtle changes over the course of the lactation period (Ballard & Morrow, 2013). Across species, maternal nutrition is essential for adequate, healthful milk production, and ultimately, the health of the offspring. There are little data currently available regarding mature milk composition during the transitional period following the first several weeks postpartum in sheep.

Lamb Health

Lamb health may be drastically impacted by the changes in lactation resulting from poor maternal nutrition. Lambs from RES fed ewes had increased 24 hour serum IgG concentrations when compared with lambs from CON and HI ewes, due to a significant effect of maternal nutrition status; maternal overnutrition resulted in reduced serum IgG concentrations (Hammer et al., 2011). The difference in IgG absorption is independent of maternal colostrum production, as lambs were fed colostrum replacer to achieve 10 g of IgG per unit of body weight (Hammer et al., 2011). However, variation in efficiency of absorption is suggested to affect the success of passive immune transfer (Alves et al., 2015). Without adequate IgG transfer resulting in sufficient passive immunity, lambs may develop a variety of illnesses, which may ultimately result in death.

A higher mortality rate was associated with lambs born to ewes with lower colostrum IgG concentrations (Tabatabaei et al., 2013). Hammer et al. (2011) conducted a study in which ewes were placed into one of two experiments, one with adequate selenium (ASe) supplementation or high selenium (HSe) supplementation, with three factorial treatment arrangements, 60% (RES), 100% (CON), and 140% (HI) of NRC requirements, resulting in the following treatment groups: ASe-RES, ASe-CON, ASe-HI, HSe-RES, HSe-CON, and HSe-HI. Lambs from ASe-RES and HSe-HI ewes were treated more frequently for respiratory and gastrointestinal illnesses and lambs from ASe-HI ewes had the highest mortality rates from birth to weaning when compared to other groups. The least morbidity occurred in single lambs born to ASe-RES ewes, while the greatest morbidity occurred in twin lambs born to ASe-RES ewes. Changes in enterocyte maturation of lambs from RES and HI ewes may alter the period of nonspecific absorption and cause altered IgG concentrations. The results of this study provide evidence that maternal diet

impacts serum IgG concentrations in neonatal lambs, which rely on adequate passive transfer of immunoglobulins and adequate nutrition for survival.

Methods of Colostrum and Milk Evaluation

Total milk solids provide a quality assessment of colostrum and milk composition. Utilizing a refractometer to determine colostral IgG concentrations and total milk solids is a practical and beneficial method for estimated quantification. Brix refractometers measure sucrose concentrations in liquids, and when used in non-sucrose containing liquids, the percentage Brix (%Brix) estimates the total solids percentage of the liquid (Deelan et al., 2014). Brix refractometry provides a strong estimate of IgG concentrations in calf serum, and is a practical and beneficial method to estimate good and poor colostral IgG concentrations in both maternal colostrum and neonate serum (Hasan et al., 2016; Deelan et al., 2014). An assessment of passive transfer of maternal immunoglobulins has shown that a Brix percentage is highly correlated with IgG concentrations (Deelan et al., 2014). In pigs, Brix values < 20% were correlated with a very low IgG concentration (14.5 mg/mL), a value not expected in early colostrogenesis (Hasan et al., 2016). Brix refractometry therefore allows for fast and efficient identification of animals producing poor colostrum on-site at farms, without the time and cost needed to conduct further laboratory analysis.

Refractometers have been found to underestimate the colostral IgG concentration when compared to concentrations determined by single radial immunodiffusion (SRID) (Chigerwe & Hagey, 2014). Single radial immunodiffusion is a highly accurate way to measure IgG concentrations in colostrum and serum, although it is time-consuming and expensive, as it requires trained laboratory technicians and 18 to 24 hours to establish results (Tabatabaei et al., 2013; Deelan et al., 2014). Both SRID and enzyme-linked immunosorbent assays (ELISA) are

considered to be the gold standard for IgG measurement in colostrum and serum (Deelan et al., 2014; Alves et al., 2015). Advantages to ELISA include a lower cost, less time to complete the assay, and the ability to quantify IgG concentrations in larger numbers of samples at one time (Alves et al., 2015). Sow colostrum evaluated utilizing both Brix refractometry and ELISA resulted in a moderate correlation of IgG concentrations ($r = 0.63$, $P < 0.001$; Hasan et al., 2016). Due to the high viscosity of colostrum, a preparation of the sample is necessary prior to running the ELISA, with documentation of up to a 1: 1,000,000 dilution being described in the present literature (Alves et al., 2015; Hasan et al., 2016).

Conclusion

Overall, poor maternal nutrition has a negative impact on the growth and metabolism of lambs (Hoffman et al., 2016; Hoffman et al., 2017; Reed et al., 2014), and the quality and quantity of colostrum and milk components, including protein, fat, and IgG antibodies (Hammer et al., 2011; Swanson et al., 2008; Hernandez-Castellano et al., 2015; Alves et al., 2015; al-Sabbagh et al., 1995). Recent studies have evaluated the effects of poor maternal nutrition on passive transfer of immunity in neonatal lambs (Hammer et al., 2011; Hernandez-Castellano et al., 2015; Alves et al., 2015; Swanson et al., 2008; Tabatabaei et al., 2013). Current data is available on the influence of the stage of lactation on milk composition in sheep (Pavic et al., 2002). Yet, there is no evidence of data in the current literature on the effects of poor maternal nutrition on the quality of transitional milk in the early lactation period in sheep. Thus, we hypothesized that maternal undernutrition would increase colostral IgG concentrations and overnutrition during gestation would reduce colostral IgG concentrations. Additionally, we hypothesized that maternal undernutrition would improve colostrum and milk density and quality

during early and transitional lactation, based on total solid concentrations, when compared to control and over-fed ewes.

Materials and Methods

Animals and Sample Collection

All animal procedures were reviewed and approved by the University of Connecticut's Institutional Animal Care and Use Committee (protocol number: A19-018).

For this project, multiparous Dorset ewes (n = 44) were estrous synchronized with intravaginal progesterone-controlled drug release devices followed by Lutalyse (Pfizer Animal Health, New York, NY). Each ewe was then bred to one of 3 rams. The breeding dates were recorded. The ewes were assigned to individual pens at day 20 of gestation. Pregnancy was confirmed using ultrasound (Jones et al., 2016). Each ewe was randomly assigned one of three diets: restricted (RES; 60%, n=15), control (CON, 100%, n=15), or over-fed (OVER; 140%, n=14) based on National Research Council (NRC) requirements for total digestible nutrients starting at day 30 of gestation. The diets were a complete pelleted feed (Pleasant View Farms, Putnam, CT) containing 74.0% TDN, 12.8% crude protein, 31.1% acid digestible fiber, and 42.1% neutral digestible fiber. Ewe bodyweight was utilized to calculate rations on a weekly basis and fed twice daily to provide 60%, 100%, and 140% of NRC. Separation of ewes in individual housing ensured proper feed intake of each animal. After parturition, ewes were transitioned to second cutting hay and pelleted sheep grain (Blue Seal, Muscatine, IA) to meet 100% of NRC requirements for TDN. Lambs had ad lib access to creep feed (Blue Seal, Muscatine, IA).

Colostrum samples were collected from each ewe within 24 hours of parturition. Milk samples were collected on days 3 and 21 postpartum from each ewe. Approximately five

milliliter samples were collected at each time point. Brix refractometry was performed, after which samples were aliquoted, and stored at -20ºC until use.

Refractometry Analysis

The concentration of dissolved solids were measured in colostrum and milk samples using an Anpro portable Brix refractometer. The optical refractometer had an upper limit of 1.120. Therefore, those samples that had a reading that surpassed 1.120 were assigned a measurement of 1.120 for analysis.

Immunoglobulin G (IgG) ELISA

Immunoglobulin G concentration was measured in colostrum samples according to manufacturer's recommendations (Sheep Immunoglobulin G, IgG ELISA, Cusabio, Houston, TX). All reagents were brought to room temperature (18-25ºC) for 30 minutes prior to use. Colostrum samples were diluted 1:2 with distilled water to form a stock solution. Stock solution was then diluted 1:3,125 with distilled water to be used for sample evaluation. Fifty µL of standard or sample was added per well, in duplicate wells. Fifty µL of HRP-conjugate was added to each well, excluding the blank, immediately. Wells were mixed and incubated for 30 minutes at 37 ºC. Each well was aspirated and washed with 200 µL of wash buffer five times. Ninety µL of TMB substrate was added to each well and the plate was incubated for 20 minutes at 37 ºC. Fifty µL of stop solution was added to each well and optical density of each well was measured using a microplate reader set to 450 nm.

Statistical Analysis

Data were analyzed using the PROC MIXED procedure (SAS Institute, Inc., Cary, NC) with main effects of time postpartum, treatment, and the interaction of time postpartum and treatment. Covariate structures were chosen based on the lowest Akaike Iteration Criterion value for maternal diet (RES, CON, OVER), time point (d 0, d 3, d 21), and their interaction. The PDIFF statement was utilized to determine the differences between means. Data are reported as mean ± SEM, with $P < 0.05$ considered significant and $0.05 < P < 0.10$ considered a tendency.

Results

Refractometry Specific Gravity Analysis

Total solid concentrations decreased significantly over time ($P < 0.0001$). Total solids were 7.9% and 8.6% lower at d3 and d 21, respectively, compared with d 0 (d 0: 1.15 ± 0.013, d 3: 1.06 ± 0.002, d 21: 1.05 ± 0.001; $P < 0.0001$). There was no observed effect of poor maternal nutrition on colostrum or milk solid concentrations ($P = 0.56$). There were no observed effects of the interaction of time point and treatment ($P = 0.35$).

Immunoglobulin G (IgG) ELISA Analysis

Colostral IgG concentrations varied significantly between RES, CON, and OVER treatment groups (RES, n = 10: 98.99 g/L ± 15.76 g/L, CON, n = 10: 154.05 g/L ± 21.08 g/L, OVER, n = 10: 173.14 g/L ± 12.30 g/L; $P < 0.0001$). Colostral IgG was lower in RES compared with CON and OVER ewes ($P = 0.03$), however there was no observed difference between CON and OVER ewes ($P = 0.37$).

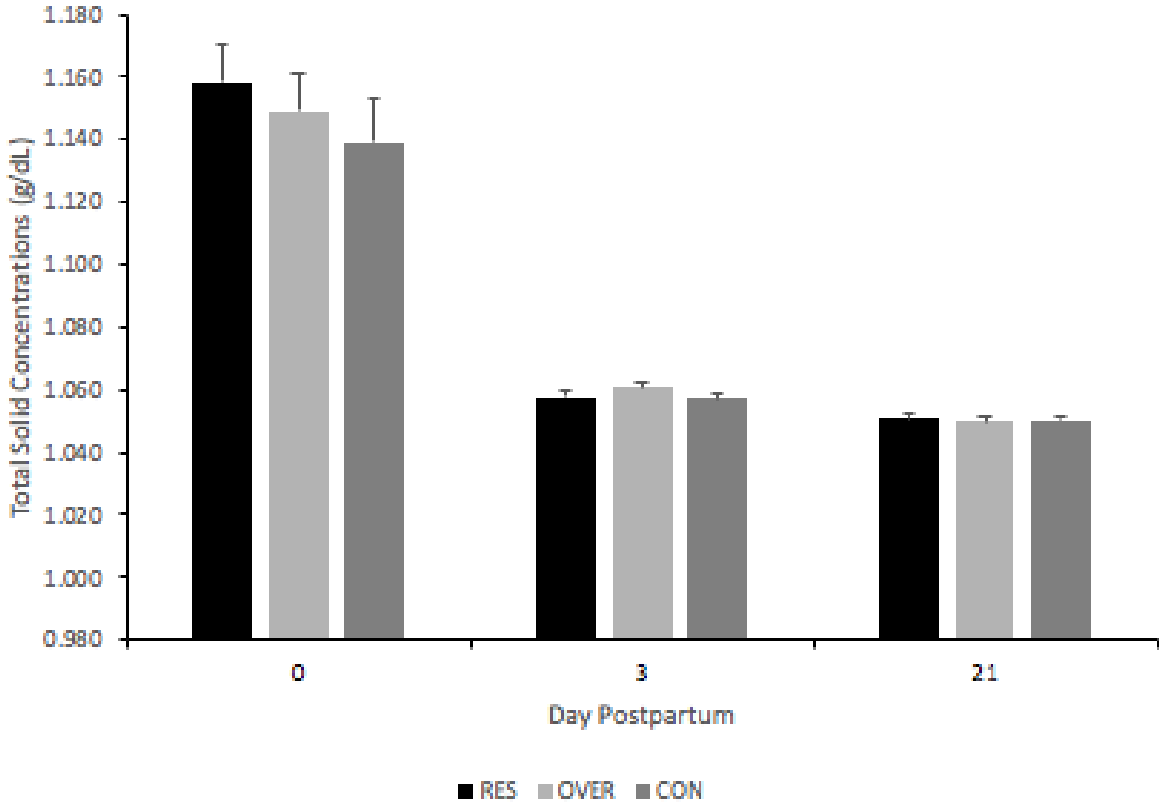

Figure 1. Total solid concentrations in colostrum and milk are affected by time postpartum but not maternal diet. Colostrum and milk samples were collected on d 0, 3, and 21 postpartum and total solids were measured via refractometry. Day postpartum significantly affected total solid concentration ($P < 0.0001$). Maternal diet did not significantly alter total solid concentrations at d 0, 3, or 21 postpartum ($P = 0.56$). There were no effects of interaction of day postpartum and treatment on total solid concentrations ($P = 0.35$).

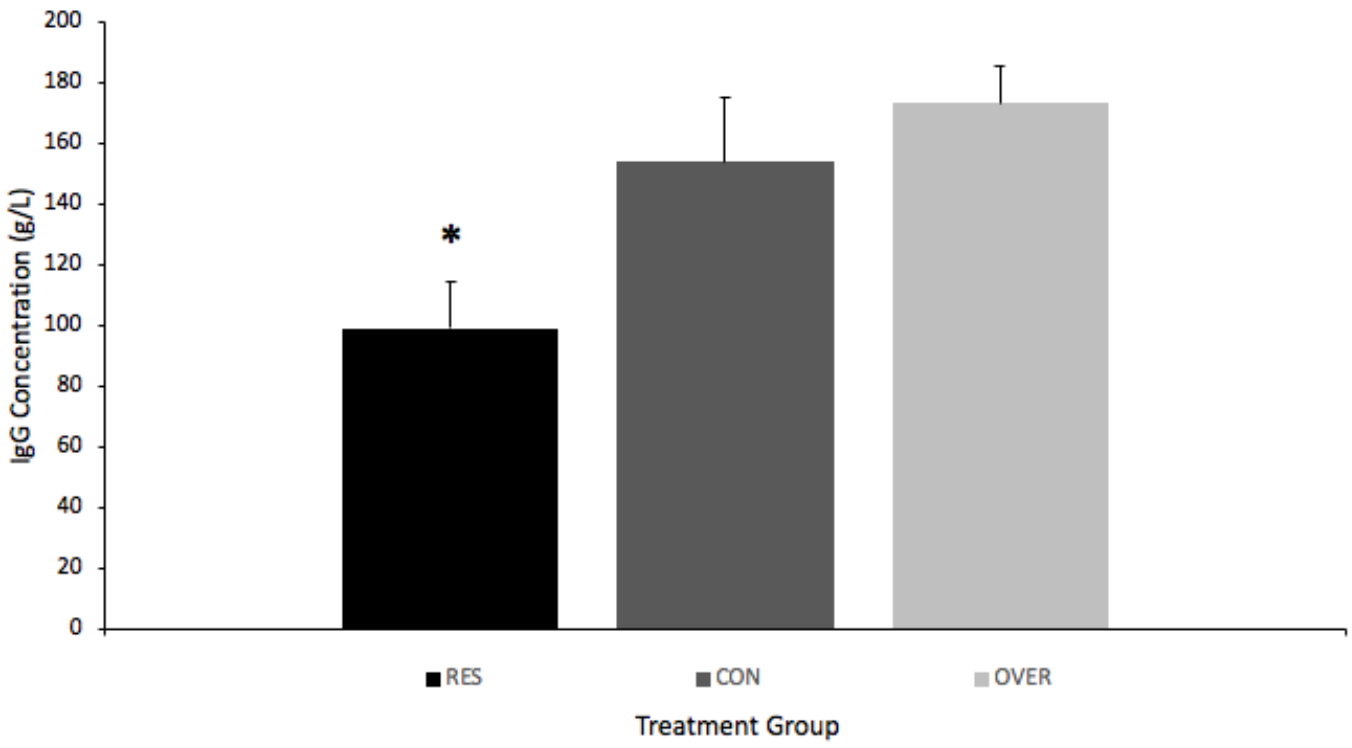

Figure 2. Colostral IgG concentrations are significantly affected by maternal diet. Colostrum samples collected within 24 h of parturition were analyzed via enzyme-linked immunosorbent assay (ELISA). Treatment group significantly affected colostral IgG concentrations ($P < 0.0001$). Colostral IgG concentrations were significantly impacted by treatment group (RES, n = 10: 98.99 g/L ± 15.76 g/L, CON, n = 10: 154.05 g/L ± 21.08 g/L, OVER, n = 10: 173.14 g/L ± 12.30 g/L; $P < 0.0001$). Colostral IgG was reduced in RES ewes when compared with CON and OVER ewes ($P = 0.03$). There was no observed difference between CON and OVER ewes ($P = 0.37$). * Indicates RES differs from CON and OVER, $P < 0.05$.

Discussion

Total solid concentrations were measured in colostrum and milk samples collected from restricted-fed, control-fed, and over-fed dams at days 0, 3, and 21 postpartum. Time point of lactation significantly impacted total solid concentrations observed in colostrum and milk samples. Ballard & Morrow (2013) note the drastic change in milk composition during the first month of lactation in humans, which is in agreement with the present study in sheep. The data support the notion that there is a drastic change in composition of milk during early lactogenesis, even in a different mammalian species.

However, maternal diet did not have a significant effect on total solids concentrations at any time point. Within the current literature, the maternal nutritional plane has significantly impacted nutritional composition of colostrum and milk in sheep (Hammer et al., 2011; al-Sabbagh et al., 1995). In Rambouillet ewes, fed restricted or over diets during gestation, total butterfat, protein, lactose, and solids but not fat were reduced compared with CON ewes (Swanson et al., 2008). Our data may differ from these significant nutritional reductions due to variations in sample analysis, including the use of refractometry to measure total solid concentrations rather than quantitatively evaluating each essential nutrient.

IgG concentrations of colostrum samples collected from restricted-fed, control-fed, and over-fed dams within 24 hours of parturition varied significantly by treatment group. Colostral IgG concentrations begin to decline drastically 2 h after parturition, with further decline between 6 h and 14 h postpartum (Moore et al., 2015), which may explain the significant change of refractometry data between days 0, 3, and 21 postpartum. Due to the incredibly rapid decline of IgG concentrations in colostrum, differences in colostral IgG concentrations may also be present

due to variation in collection time between animals. Further consideration should be taken to evaluate colostrum at various time points within the first day postpartum, in order to determine the effects of collection time on colostral IgG concentrations and at what time points IgG concentrations are reduced linearly or exponentially during the first 24 hours postpartum.

Additionally, Tabatabaei et al. (2013) determined that breed had a significant effect on colostral IgG concentrations. Shaul ewes on a traditional maternal diet during gestation produced colostral IgG concentrations ranging from 25 to 100 mg/mL, similar to Rambouillet and Columbia sheep on traditional maternal diets during gestation. Lori Bakhtyari ewes on a traditional maternal diet during gestation produced significantly lower colostral IgG concentrations, ranging from 7.35 to 90.65 mg/mL, similar to reported values of Suffolk sheep. Colostral IgG concentrations from ewes of the Dorset breed may vary from breeds previously used in similar studies. Dorsets are hardy dual-purpose animals, similar to Rambouillet, Suffolk, and Columbia sheep. In addition to the different purposes of each breed, environmental conditions and different genetic lineages may contribute to variations noted in lactation, including colostral IgG concentrations. Interbreed variation may also result in polymorphisms of the neonatal Fc receptor gene, resulting in differences in the transfer of IgG from serum to colostrum.

Further evaluation of specific nutrients critical to neonatal health and development, particularly fat and protein concentrations, in colostrum and milk samples is warranted, in order to determine the effects of breed and maternal diet on lactation. There is evidence that ewe nutritional plane during gestation results in inadequate passive transfer of IgG, increasing lamb morbidity and mortality (al-Sabbagh et al., 1995; Swanson et al., 2008; Hammer et al., 2011; Alves et al., 2015). In addition, Hammer et al. (2011) observed decreased birth weight in lambs

from RES and HI ewes when compared to those from CON ewes, and increased 24-h IgG concentrations in lambs from RES ewes. Thus, further research and evaluation of serum IgG concentrations collected from lambs at days 0, 3, and 21 after birth are warranted, to evaluate the passive transfer rates of immunoglobulins to lambs from restricted-fed, control-fed, and over-fed dams. Overall, future investigations will study the impacts of maternal diet on postnatal lamb health and mortality, as a result of milk quality and adequate passive transfer rates of IgG postnatally.

Conclusion

In conclusion, poor maternal nutrition did not have a significant effect on total solids concentrations during early stages of lactation. Nutritional treatment group did have a significant effect on colostral IgG concentrations, with significant reductions in IgG concentrations in the colostrum of RES ewes when compared to CON and OVER ewes. In agreement with previous literature (Pavic et al., 2002; Ballard & Morrow, 2013), time point postpartum did have significant effects of total solid concentrations postpartum. Previous data have shown that maternal diet during gestation impacts neonatal health and the ability of the neonate to acquire passive immunity of immunoglobulins via colostrum (Hammer et al., 2011). Further analysis is warranted to determine the success of passive transfer in lambs and generational effects of compromised colostrum and milk quality

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Appendices

Appendix I: Sheep Immunoglobulin G (IgG) ELISA Protocol

This procedure is adapted from Hasan et al. (2016) and describes a modified method outlined Sheep Immunoglobulin (IgG) ELISA (Cusabio, Houston, TX) to enable reading on a microplate reader set to 450 nm.

Supplies:

- Cusabio Sheep Immunoglobulin (IgG) ELISA (Cusabio; Catalog #CSB-E14400Sh)
- Colostrum sample
- Microplate reader equipped for spectral plate reading at absorbance 450 nm
- 2 mL microcentrifuge tubes
- 0.5 mL microcentrifuge tubes
- Adjustable micropipettes (50 to 1000 µL) and tips
- Multichannel pipette
- Deionized or distilled water
- Wet ice

Procedure:

1. Pre-assay steps
    a. Label two 2 mL microcentrifuge tubes per sample on tube top
    b. Label 96 well plate with each standard and sample's respective identification in duplicate
    c. Perform colostrum sample dilution
        i. 1:2 dilution of sample to deionized or distilled water
        ii. 1:3,125 dilution of 1:2 stock solution to deionized or distilled water
        iii. Keep samples on ice
    d. Prepare reagents
        i. HRP-conjugate
            1. Centrifuge HRP-conjugate to mix
            2. Dilute 10 µL of HRP-conjugate to 990 µL of HRP-conjugate diluent
        ii. Wash buffer
            1. Warm to room temperature and mix
            2. Dilute 20 mL of wash buffer concentrate to 480 mL of deionized or distilled water
        iii. Standard
            1. Centrifuge standard vial at 6000 to 10000 rpm for 30 s
            2. Allow standard to sit for 15 minutes
            3. Label five 0.5 mL microcentrifuge tubes zero through five
            4. 150 µL of sample diluent into each microcentrifuge tube

5. Standard (240 µg/µL) serves as high standard, add 150 µL of standard to next microcentrifuge tube
6. Prepare two-fold dilution series, sample diluent serves as zero standard (0 µg/µL)

2. Performing the Assay
    a. Set blank well with no solution
    b. Add 50 µL of standard or sample in respective wells
    c. Add 50 µL of HRP-conjugate to each well (not blank)
    d. Mix and incubate for 30 minutes at 37 ℃
    e. Aspirate each well
    f. Add 200 µL of wash buffer
        i. Allow to sit for two minutes and remove
        ii. Complete five total washes
    g. Add 90 µL of TMB substrate
        i. Protect from light
    h. Incubate for 20 minutes at 37 ℃
    i. Add 50 µL of stop solution, mix
    j. Insert plate in to microplate reader within 5 minutes
        i. 450 nm
        ii. Determine optical density
3. Export data for analysis