INFLUENCE OF MARE BODY COMPOSITION ON COLOSTRUM QUALITY

A Thesis

by

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ABSTRACT

Twenty-Two Quarter Horse mares, fed 100% NRC recommendations, and their offspring were used to evaluate various body composition measurements as indicators of colostrum quality. Starting 45 d pre-partum mares were evaluated weekly using the Body Condition Score system, ultrasonic measurements of rump fat, rib fat, loin area and intramuscular fat, and Westervelt's equation to determine percent body fat. Post parturition colostrum samples were collected by hand at 0, 6, 12, 24 and 36 h post-partum and evaluated for protein, fat, lactose, somatic cell count, urea nitrogen, acetone, total solids, non-fat solids, IgG, IgA and IgM. Foal blood was collected via jugular venipuncture at 0, 12 and 24 h post-partum to determine the failure or success of passive transfer. Colostrum composition at 0 h was similar to previous research. Immunoglobulin isotype concentrations at 0 h were lower than previous research. Data from 6, 12, 24, and 36 h provide further insight regarding the rapid change from colostrum to mature milk, as there is significant change from 0 to 12 h ($P<0.0001$). While no single measurement provided consistent correlations to colostrum quality or composition, the trend was that fatter horses produced less protein and non-fat solids, and more lactose and fat. Furthermore, the combination of rib eye area at the 13th/14th ribs, fat at the 13th/14th ribs and rump fat was found to be the best indicator for body condition and/or overall fatness ($P<0.001$; $R2=0.80145$).

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Passive transfer

Passive transfer of immunity is a vital part of the equine neonate's life. The equid possesses a diffuse placenta which is characterized by specialized chorionic villi called micro-cotyledons. It is also referred to as an epitheliochorial placenta, characterized by the complete separation of the fetal membranes (chorion) and the maternal membranes (endometrial epithelium) (Senger, 2003; Chucri et al., 2010). This type of placenta lacks immunoglobulin transporting proteins and therefore fails to allow immunoglobulins to transfer from the dam to the foal across the placental barrier, resulting in the neonates' hypogammaglobulinemic state. Since foals are born with a naïve immune system, postnatal immunity transfer occurs through the gut wall after ingestion of colostrum, which is believed to need to occur within 24-36 h after birth, before the gut wall closes (Langer, 2009; Hurley and Theil, 2013). Absorption of immunoglobulins through the gut wall in the equine neonate is thought to be similar to that of the bovine neonate, in that colostrum in ingested by the neonate, it is taken up by villous epithelial cells within the ileum, transferred to the lacteals which pass the macromolecules on into the lymphatics, which then transfers into the circulation of blood (Jeffcott, 1972). Further investigation utilizing an inorganic compound additive found that maximum absorption occurred 3 h after birth and displayed a linear decline until absorption fell under 1.0% around 20 h

after birth (Jeffcott, 1974). The cessation of absorption of macromolecules across the gut wall occurs when the non-selective enterocytes responsible for the pinocytosis of immunoglobulins are replaced by mature enterocytes coated in digestive enzymes with the purpose of water, sugar, protein, lipid and vitamin uptake (Giguere and Polkes, 2005).

Failure of passive transfer (FPT) is characterized by less than 400 mg of IgG per deciliter of whole blood 12-16 h after birth. Partial failure is characterized by 400-800 mg of IgG per deciliter of whole blood and passive transfer is considered successful when there is greater than 800 mg of immunoglobulins per deciliter of whole blood after 12-16 h. Failure of passive transfer can occur when the mare produces low quality colostrum and/or when the ingestion of colostrum is delayed or limited (Chavatte-Palmer et al., 2001). Since foals are born in a hypogammaglobulinemic state, they are more susceptible to bacterial and viral infections (Wagner, 2006). Research has shown that FPT foals are more likely to become ill and less likely to survive when compared to foals which receive adequate protection through consumption of immunoglobulin-rich colostrum. One such study of 597 hospitalized foals found a proportional association between low IgG levels and mortality (Liepman et al., 2015). A common way of testing for FPT in foals in the equine industry is to use a SNAP Foal IgG Test Kit (IDEXX), however research has shown that while this provides some useful information when evaluating the foal, the performance of the test is impaired due to low specificity (Metzger et al., 2006). Further analysis can be done with an enzyme-linked immunosorbent assays (ELISAs), which can be used to evaluate concentrations of

isotypes and sub-isotypes at more specific level rather than in wide ranges of combined immunoglobulins like the SNAP test.

Immunoglobulins

Passive transfer of immunity is made possible by the high concentrations of immunoglobulins in colostrum, the first milk produced by the mother (Langer, 2009). Immunoglobulins can be classified into 5 isotypes: IgG, IgA, IgM, IgE and IgD, and consist of similar structures composed of four subunit polypeptides, two of which are identical heavy chains and two of which are identical light chains (Sheoran et al., 2000). Recent characterization of equine immunoglobulins has shown that horses have primarily λ light chains and γ heavy chains, which all have distinct immune response functions (Wagner, 2006). IgG is characterized by γ heavy chains and is mainly a secondary response to infection; as it acts by finding a phagocyte receptor to initiate opsonization, or targeting bacteria for destruction. IgM contains μ heavy chains, is the first antibody built during an immune response, and is responsible for agglutination. IgA is characterized by its α heavy chains and protects the mucus membranes. IgE contains ε heavy chains and protects against parasites. IgD is characterized by δ heavy chains and its functions are largely unknown in the horse (Thermofisher, 2017). The most prevalent immunoglobulin isotypes in equine colostrum are IgG, IgA and IgM (Sheoran et al., 2000; Hurley and Theil, 2013). In a study of 7 Welsh ponies, 27 mixed-breed horses and 5 quarter horse mares, IgGb was the most abundant isotype in colostrum, and IgA was the most abundant in mature milk (Sheoran et al., 2000). Wagner, using the new nomenclature for immunoglobulin isotypes and sub-isotypes, found the concentrations

of immunoglobulins in colostrum to rank (greatest to least) IgG4, IgG1, IgG3+5, IgA, IgM and IgG6 with no data for IgE (Wagner, 2006). Immunoglobulins make up more than 40% of protein in equine colostrum, 30% consisting of IgG and 10% of IgG(T) (Rouse et al. 1970). Langer (2009), determined that colostrum contained 191 g of protein per kg, suggesting that colostrum contains large concentrations of immunoglobulins, compared to animals that pass immunity to their offspring in utero. This is consistent with earlier research in which it was determined that colostral specific gravity was significantly correlated (r=0.9) with colostral immunoglobulin concentrations (LeBlanc et al., 1986)

Immunoglobulin concentrations have a marked decline from colostrum collected immediately after parturition compared with colostrum collected 9-24 h after parturition, IgG and IgG(T) dropped from 30 and 10 % to 7 and 2 % respectively (Rouse and Ingram, 1970). A study of Thoroughbred mares determined the immunoglobulin portion of protein to drop from 43.1 g/l to 11.0 g/l within 12 h after parturition (Curadi et al., 2000). Wagner, who compared data from various studies, found a sharp decline in immunoglobulins from colostrum to milk. IgG4 dropped from 183 mg/ml to 3.1 mg/ml, IgG1 from 82 mg/ml to 1.6 mg/ml, IgG3+5 from 44 mg/ml to 0.8 mg/ml, IgG6 from 0.3 mg/ml to 0.06 mg/ml, and IgA from 9 mg/ml to 0.3 mg/ml (Wagner, 2006). The data show that there are obvious differences between colostrum and milk, which are apparent fairly soon after parturition. This means that there is a short window of time in which colostrum is available for the foal before the lactational change to milk. The possible

consequences of this change include the foals' inability to receive immunity from the mare if the it fails to suckle promptly after birth.

Evaluation of colostrum quality

In the equine industry, it is standard procedure to estimate the immunoglobulin concentration of colostrum to determine the potential of failure of passive transfer (FPT). Since enzyme-linked immunosorbent assays and radial diffusion are not simple, cheap or readily available, foaling facilities rely on devices such as a Brix Refractometer or Colostrometer. The Brix Refractometer is a hand-held prism used to determine the amount of total solids in a liquid. A Colostrometer is used to evaluate the specific gravity of a liquid. These tools have been studied in correlation with immunoglobulin concentrations and have been determined to be useful tools for on-farm evaluation of colostrum. After looking at total protein, specific gravity, viscosity, refractive index and latex agglutination in comparison to IgG measurements from radial immunodiffusion, it was determined that using a specific gravity to estimate IgG concentrations was an appropriate method for on farm evaluation due to high correlation between the two (Waelchli et al., 1990). Other authors agreed that using a sugar or alcohol refractometer, which measures the total solids of a liquid, provides a more objective measurement compared to colostrum color or consistency and could be a useful tool for the equine industry (Chavatte et al., 1998). Further investigation found strong correlations between the refractometry index of colostrum and milk with immunoglobulin concentrations determined by radial immunodiffusion, further validating its use in on-farm colostrum evaluation (Korosue et al., 2012). The use of the Brix Refractometer has been validated

for evaluation of bovine colostrum, as seen in one study spanning 7 facilities in 4 states and compared against radial immunodiffusion in which the authors concluded that the Brix Refractometer was an inexpensive, rapid and satisfactorily accurate tool for the use of evaluating IgG in colostrum (Quigley et al., 2013). The Brix Refractometer has also been validated for use for porcine colostrum, when a study of 153 sows compared the refractometry results against that of enzyme-linked immunosorbent assays found a correlation of $r=0.63$, $P<0.001$, which reiterates the results found in bovine studies (Hasan et al., 2016). The Colostrometer, also referred to as a hydrometer, is a glass cylinder that floats in colostrum, or is filled with colostrum and floated in water, to determine specific gravity which is highly correlated with immunoglobulin concentrations. Colostrometers were first validated for use with bovine colostrum, when specific gravity correlated highly with immunoglobulin G (Mechor et al., 1992). The Colostrometer was then adapted and validated for use in evaluating caprine colostrum, resulting in a correlation of $r=0.99$ between specific gravity and immunoglobulin concentrations (Rudovsky et al., 2008). Typically, bovine colostrometers are bought and used in the equine industry due to being inexpensive and readily available.

Another common procedure used in the equine industry, by veterinarians and breeders alike, is to test foal blood for immunoglobulin concentrations to determine level of passive transfer. As early as 1988 these types of kits were tested for accuracy; one study tested 88 samples using a semi quantitative IgG assay assessing passing (>800 mg/dl), partial failure (400-800 mg/dl) and failure (<400 mg/dl) of passive transfer had correctly analyzed all but one sample when checked against radial immunodiffusion

(Bertone et al., 1988). More recent research has evaluated the refractometer to estimate total protein content of foal blood and found strong correlations with radial immunodiffusion lending credibility to the use of a refractometer as an on-farm method for evaluating passive transfer (Korosue et al., 2012). The test currently used by a good portion of the equine industry is the SNAP Foal IgG Test (IDEXX), which was validated in 2002, when samples from 42 foals were tested and compared against single radial immunodiffusion, and was found to have acceptable accuracy with low and high concentrations (80% and 89% respectively) but was found to test lower for intermediate concentrations (Pusterla et al., 2002).

Colostrum composition

In addition to being the primary source of immunity for the foal, colostrum and mature milk are the foal's main source of nutrients and hydration for the first several months of life. Lactation in the mare changes from colostrum to milk within the first few days after parturition and is characterized by several changes in composition. The total solids and fat content decreases from colostrum to transitional milk to normal milk, and vitamins A, D, K and C were found to be 1.4-2.6 times the concentration in equine colostrum than found in normal milk (Csapó et al., 1995). Protein was found to decline dramatically from 14.9% collected immediately after parturition (0-3hrs) to 5.05% collected between 4 and 8 h after parturition, to 3.85% between 9 and 24 h after parturition (Rouse, 1970). Similar results were seen by Salimei et al. (2002) who showed that all levels of major components changed drastically in pre-suckle colostrum and post suckle colostrum. Within the first 24 h total solids dropped from 202.5 g/L^{-1} to 119.2

g/L^{-1} and protein dropped from 160.1 g/L^{-1} to 33.9 g/L^{-1}. Fat and lactose on the other hand increase from 7.2 g/L^{-1} to 24.9 g/L^{-1} and 34 g/L^{-1} to 60.4 g/L^{-1}, respectively (Salimei et al., 2002). Langer (2009) found significant differences in protein, fat and carbohydrate concentrations between colostrum and mature milk, changing from 191 g/L^{-1}, 7 g/L^{-1} and 46 g/L^{-1}, to 25 g/L^{-1}, 20 g/L^{-1} and 65 g/L^{-1}, respectively. A study of Thoroughbred mares also provided data showing that fat and lactose increase from 1.73% and 5.55% to 2.61% and 6.72%, respectively, over the course of 4 mo. Concurrently, protein and non-fat solids dropped from 3.15% and 9.45% to 1.8% and 9.27% (Hoffman et al., 1998).

Mature milk is produced after colostrum has been depleted which generally occurs within 24 h after parturition, is unique to each species, and is the primary source of nutrition for the animal's offspring. In the case of the equid, milk is the only required feedstuff for the foal until about 3-4 months of life, at which time additional sources of nutrition are required. Gibbs et al, (1982) found the largest mean daily milk yield was collected approximately 30 d postpartum, and that it steadily regressed after that point. Over 150 d of lactation the mares produced an average of 2.13% body weight consisting of 10.5% solids, 2.14% protein and 1.3% fat (Gibbs et al. 1982). The control mares in another study, maintained at an average of BCS of 6 compared to obese BCS 8+ mares, produced an average of 14.4 kg/d, with an average of .3% fat, 1.56% protein and 9.74% total solids (Kubiak et al., 1990) Eight Murgese mares, a light-bred breed descended from Arabians, produced an average of 4.91 kg of milk per hand milking and 7.69 kg of milk per machine milking session, both of which were performed twice a day during

their respective treatments periods. During hand milking, the authors recorded the mature milk to consist of 1.06% fat, 7.04% lactose, 1.85% crude protein and 3.81$\log_{10}$ somatic cells per ml (Caroprese et al., 2007). A study of Polish cold-blooded mares during late stage lactation reported a mean of 4.2 g/L^{-1} and low proportions of saturated fatty acids C14:0, C16:0 and C18:0 and high proportions of C8:0, C10:0 and C12:0 (Markiewicz-keszycka et al., 2014). A study of Thoroughbred mares reported that the mares produced mature milk consisting of 2.08% fat, 2.23% protein, 6.52% lactose and 9.5% non-fat solids (Hoffman et al., 1998).

Body condition

Body condition and composition of the equine is evaluated most frequently using Henneke's Body Condition Score (BCS) system which was developed in 1983. The BC system is a system ranging from 1 to 9, with 1 being emaciated and 9 being obese. The areas of the horse evaluated to determine body condition score are the neck, withers, back or loin, tail head or rump, shoulder pocket and ribs (Appendix Figure A1). Low body condition scores are indicated by visible bone structure of the neck, prominent bone structure of the withers, lack of muscle and fat across the back and loin, prominent tail head and visible ribs. High body condition scores are given when there is ample fat cover, a crease along the back and loin, sponginess behind the shoulder and around the tail head, difficulty feeling the ribs and in extreme cases patches of fat along the body (Henneke et al., 1983). While useful in the industry, the BCS system can be subjective and other methods of determining body composition and animal fattiness have been developed. One such method is the use of ultrasound to measure for fat thickness over

the rump. Westervelt et al. (1973) used ultrasonic measurements to evaluate fat content by measuring fat thickness halfway from point of hip to point of buttock, 5 cm from the midline, via ultrasound and then harvesting the equine carcasses and measuring the extracted fat. The authors developed an equation, $Y = 8.64 + 4.70X$, where X= rump fat in centimeters, to calculate percentage of body fat. The authors determined that the use of ultrasonic measurements is an accurate and objective method of predicting total body fat of horses and ponies (Westervelt et al., 1976). A furth study was conducted in 2004 to validate the use of ultrasonographical measurements when evaluating equine body composition. Gentry et al. measured subcutaneous fat at the tail head, rump, 13th rib and withers, and found strong correlation coefficients for each, suggesting that ultrasonographical measurements are accurate in estimating equine body composition (Gentry et al., 2004). Silva et al. (2015) further investigated the idea of using ultrasonography for the evaluation of equine body composition and found a strong relationship between fat measurements at the 3rd lumbar vertabra and body condition scores, confirming that ultrasonic measurements may provide a more objective method of fat evaluation. Other methods of measuring adiposity have been tested, such as Cresty Neck Scores which measure the amount of fat palpated along the top of the neck ranging from 0 to 5, and height, length, girth, abdominal circumference, neck crest height and neck circumference ratios (Carter et al., 2008). In this study girth circumference to height ratio was determined to have the strongest correlation with BCS (r^2=0.68, $P<0.001$) and neck crest height and neck circumference to height ratio had the strongest association with Cresty Neck Scores ($r^2>0.50$, $P<0.001$). The authors also found that

girth to height ratio was the best predictor for the assessment of adiposity among of the measurements evaluated and felt that this is a more standardized method over BCS, however this method appears to be less accurate than ultrasound measurements and has been utilized less in research (Carter et al., 2008). Despite further research, the most commonly used method of evaluating body composition in the equine industry is the BCS system as it is simple to do and requires no equipment.

Broodmare condition and nutrition

The most appropriate condition for a broodmare is in the 6 to 7 range which allows for some leeway in terms of potential weight loss during negative energy periods. Early data show that broodmares with low body condition had lower conception rates, longer intervals postpartum, and required more cycles per conception when compared to the mares with greater body condition scores (Henneke, 1982). A study of 32 lactating mares showed a decrease in body fat during lactation, which supports the theory that stored body fat is a primary source of energy during lactation and as well as the idea that broodmares need to be on the fatter end of the BCS scale to prevent any lactational or reproductive deficits caused by emaciation (Henneke et al., 1984). In another study, low body weight of the mare resulted in further weight loss during lactation and decreased foal growth (Doreau and Boulot, 1989). More research illustrated that low body condition scores of 3-3.5 resulted in a profound seasonal anovulatory period in open mares. (Gentry et al., 2000). This could prevent early breeding dates for those trying to produce foals born in January, which is common throughout the equine industries.

At the other end of the spectrum, horses should not be fed to obesity, as that has been shown to decrease their lactational ability. One equine study showed that obesity was correlated with lower milk yield per body weight but that the composition of colostrum did not differ between control and obese mares (Kubiak et al., 1990). In other species, such as rats and humans, obesity has resulted in delayed initiation of lactation as well as early cessation. A study with rats found the young of the high fat fed rats to experience higher mortality rates compared to the offspring from control diet rats, 16.5 to 7.7%, respectively which prompted further research into the effects of obesity on lactation (Shaw et al., 1997). Rats fed a high fat diet showed latent initiation of lactation as well as low milk production resulting in poor pup growth and higher instances of pup mortality. Comparatively, rats fed low fat diets produced 50% more milk than the high fat fed rats. (Rasmussen, 2001). Based on these data, a similar study was conducted with human subjects to determine if obesity had similar effects on lactation. Over 3,800 women were analyzed over a 9-yr period, and were categorized as normal, over weight and obese according to BMI evaluation. Women in the study who were overweight or obese were significantly more likely to experience unsuccessful initiation of breast feeding as well as early cessation of breast feeding (Rasmussen, 2002). A study of 18 ewes, half fed to obesity at 150% NRC nutrient recommendation, produced data showing that maternal obesity resulted in a decrease of protein concentrations in the colostrum (Long, 2009).

Other considerations regarding the over feeding of broodmares include the increased chances of metabolic diseases in both the mare and foal. In a study of 17

Egyptian broodmares, it was found that obese and overweight mares had hyperinsulinemia, hypertriglyceridemia and hyperglycemia, metabolic conditions that can negatively affect the mare and potentially result in loss of pregnancy or loss of life (El-maaty et al., 2017). Another study showed a correlation between BCS and increased leptin levels, and that lactating mares were more likely to be hyperleptinemic than non-lactating mares of the same body condition score (Huff et al., 2008). Obesity and adiposity are risk factors, which when exacerbated by poor management, over feeding, and inadequate exercise, are highly correlated with equine metabolic syndrome. Equine metabolic syndrome is important because it can result in laminitis, a severely debilitating disease that often results in humane destruction of the animal (Frank, 2009). One review recommends that equine metabolic syndrome, pars pituitary intermedia dysfunction obesity and hyperinsulinemia be remedied prior to breeding as they increase the risk on laminitis (Galantino-homer et al., 2012).

Furthermore, obesity and fat deposition have been thought to result in infertility. In humans, obesity has been shown to result in sex hormone secretion and metabolism disorders (Pasquali, 2006). That being said, several studies show that equine obesity can result in prolonged ovulatory periods. Gentry et al. (2000), found 11 out of 12 obese mares with a body condition score of 7.5-8.5 continued to cycle or show follicular activity through the winter, and presented with higher leptin, prolactin and IGF-I levels. Similar results were found again in 2006 when obese (BCS of 7 or greater) were found to have longer estrus periods, and an increase of circulating leptin and insulin and decreased insulin sensitivity (Vick et al., 2006). Cavinder et al. (2012) investigated the

difference in fertility between fat mares (BCS 7-8) and moderate mares (BCS 5-6) but found no differences in number of days to foal heat ovulation, interovulatory interval or conception rates.

When planning a feeding regimen for pregnant or lactating mares, it is recommended that the mare's breed, age and body condition (BCS) be taken into account throughout their pregnancy and their feed adjusted accordingly in response to any changes. The mare's energy requirements can be calculated by estimating the mares maintenance requirements, milk yield and energy value of milk (Doreau et al., 1988). Burns et al. (1992), measured milk energy produced at 10, 30, 45, 60, 90, 120 and 150 d postpartum, and determined that total milk energy produced ranged from 3.1 to 7.8 Mcal/d and peaked at d 10 before declining in a linear fashion (Burns et al., 1992). Currently the NRC recommends that horses in good health ingest 30-36 kcal of digestible energy/kg of body weight depending on the type of horse, pony, light, heavy etc., with an extra 792 kcal/kg of milk produced per day (NRC, 2007). One way to calculate this is: maintenance + [(0.03* x bw x 0.792) x 4.1841)] (Harris, 2003).

Despite nutritional guidelines, attempts to negatively impact gestation or lactation through over feeding has shown varied results. Coverdale et al. (2014) found that over feeding mares at 140% NRC requirements resulted in increased glucose and insulin levels and a decrease in IgG concentration in colostrum but did not slow foal growth or result in failure of passive transfer. Cavinder et al. (2012) fed mares either at 100% or 120% NRC requirements for late gestation and results indicated that leptin concentrations in plasma from the overfed mares were greater, that the leptin

concentrations in plasma from foals out of control fed mares were greater, and that plasma concentrations of IGF-1 and cortisol were the same in both groups of foals. A study of 18 pregnant Quarter Horse mares fed with grain with 5% rendered fat showed that the mares produced milk with higher fat percentage, shorter postpartum intervals and fewer cycles per pregnancy, with similar foal birth weight and weight gains in both the control and fat fed groups (Davison et al., 1991). A similar study of Thoroughbred mares fed conventional rations or fat and fiber supplemented rations showed early milk from fat and fiber supplemented mares to consist of higher percentages of fat and protein, although over time these percentages evened out (Hoffman et al., 1998). This suggests that the requirements are not fully understood, that they may exceed the current NRC suggestions, and therefore that they should be further investigated.

Based on previous research, the following objectives were developed:

1) Identify concentrations of immunoglobulins in equine colostrum.
2) Evaluate colostrum composition changes within the first 36 hours after parturition.
3) Pinpoint the transition from colostrum to mature milk.
4) Determine the correlation, if any, between body condition, rump fat, back fat, rib fat, intramuscular fat and colostrum composition and quality.
5) Provide further justification of ultrasonic measurements as an objective method of the evaluation of body composition.

CHAPTER II

MATERIALS AND METHODS

Animals, diet and management

Twenty-Two Quarter Horse mares and their foals were used in this study. The broodmares ranged in age 5 to 23 yr (mean = 12.4 yr), had body condition scores (BCS) from 3 to 7 (mean = 5.25) and had an average body weight (BW) of 573.25 kg. They were housed in groups of 9 or less in paddocks at the Texas A&M Equestrian Center and placed in 12’ by 24’ foaling stalls as they neared parturition; criteria considered in this decision included estimated foaling date (EFD), changes in mammary secretion color, consistency, and pH. Each mare had ad libitum access to Coastal Bermuda grass hay (1.01 Mcal/lb DE, 8.73% CP) and was fed a 1.47Mcal/lb DE, 15.67% CP, 5.49% Fat pelleted feed to meet digestible energy requirements according to NRC (2007) requirements (Appendix Table A 1). Mares were kept at the same Body Condition Score throughout the project. To this end, mares were weighed weekly, at the same time every Sunday without feed restriction, and grain allowance adjusted to maintain BCS. Concentrates were provided in separate stalls to ensure each mare received the correct amount of grain, which was calculated to meet 100% NRC requirements. An hour was allotted for feed consumption and mares were then returned to their perspective dry lots. Horses used in this study were maintained under the approval of the Texas A&M

University Institutional Agricultural Animal Care and Use Committee (AUP# 2016-0364) using guidelines set forth by the Federation of Animal Science Societies.

Sample collection

Mare BW was obtained weekly beginning 45 d prior to EFD on a Cardinal Model 205 Weight Indicator (www.cardinalscale.com) to determine body weight. At the same time, each was evaluated for BCS by three graduate students trained according to Henneke et al. (1983) and the scores averaged. Rump fat thickness was measured with a MicroMaxx Ultrasound System (SonoSite Inc.) half way between the point of hip and point of buttock, 5.0 cm from the midline (Appendix Figure A 2.). The area was clipped, and 70% alcohol was applied to the skin to increase contact between the probe and skin and increase accuracy. Once every two weeks mares were scanned for rump fat, rib fat at the intercostal space between the 13^{th} and 14^{th} ribs and 17^{th} and 18^{th} ribs, loin eye area at the intercostal space between the 13^{th} and 14^{th} ribs and 17^{th} and 18^{th} ribs, and intramuscular fat by a certified sonographer and images evaluated by International Livestock Image Analysis Centralized Ultrasound Processing Lab at Designer Genes USA Inc. The rump fat scan location was 5.0 cm off the midline, half way between point of hip and buttock, rib fat, loin area and intramuscular fat measurements were taken at the intercostal space between the 12^{th} and 13^{th} ribs as well as the 17^{th} and 18^{th} ribs. Percent body fat was calculated using Westervelt's' equation, $Y = 8.64 + 4.70X$, where X equaled rump fat in cm. Colostrum and foal blood samples were collected in private foaling stalls. Colostrum was collected by hand into sterile conical vials at h 0, 6, 12, 24 and 36 after parturition. Foal blood was collected by jugular venipuncture with a

CORVAC® serum vacutainer tube with no additives and Vacuette blood collection needles at h 0, 12 and 24 after parturition. Blood was centrifuged in an Eppendorf Centrifuge 5810 (Hauppauge, NY) at 1500 g for 3 min to separate serum. Fifteen mL of colostrum from each collection were frozen in 1.5 ml vials at -80˚C. Serum from 15 ml of foal blood from each collection were also frozen in 1.5 ml vials at -80ºC until all mares reached parturition.

Sample analysis

Colostrum collected at 0 h was immediately evaluated using a Brix Refractometer and Colostrometer to determine colostrum quality via industry standard methods. Twenty-five mL of colostrum from each collection was sent to the Texas Dairy Herd Improvement Association (Canyon, Texas) to be analyzed for fat, protein, lactose, somatic cell count, urea nitrogen, acetone, total solids, and non-fat solids with a Foss Fossomatic™FC Somatic cell counter and Foss MilkoScan™FT+ for milk analysis. After all mares had foaled, frozen samples were thawed and evaluated by enzyme-linked immunosorbent assays (ELISAs) to determine IgG, IgM, and IgA concentrations according to manufacturer instructions. ELISA sets and accessories were purchased from Bethyl (Montgomery, Texas) and Immunology Consultants Inc. (Portland, Oregon). Foal blood collected approximately 24 h after parturition was used to perform a SNAP Foal IgG Test (IDEXX) to determine passive transfer.

Statistical analysis

PROC CORR, PROC REG and PROC MIXED were conducted using SAS to determine the relationships between body composition measurements: body condition score, rump fat, rib fat at the 13th and 14th intercostal space, rib fat at the 17th and 18th intercostal space, loin area at the 13th and 14th intercostal space, loin area at the 17th and 18th intercostal space, intramuscular fat and percent body fat, and colostrum composition parameters: protein, fat, lactose, acetone, urea, total solids, non-fat solids, somatic cell count, as well as total solids determined by Brix Refractometry, specific gravity determined by Colostrometer, IgG, IgA, and IgM concentrations. Changes in colostrum composition over time were also examined using linear regression in SAS (SAS, INC., 2016).

CHAPTER III

RESULTS

Evaluation of colostrum

Table 1 summarizes the composition of colostrum and milk over 36 h of lactation. The results show that protein decreased over time (P<0.0001) (Figure 1.), lactose increased over time (P<0.0001) (Figure 2.), and non-fat solids decreased over time (P<0.0001) (Figure 3.).

Table 1. Evaluation of colostrum

	h 0	**h 6**	**h 12**	**h 24**	**h 36**
Fat %	1.466 ± 0.71	2.158 ± 1.37	2.031 ± 0.74	1.972 ± 0.48	2.013 ± 0.5
Protein %	13.14 ± 5.25	9.505 ± 3.11	3.627 ± 0.94	3.545 ± 2.76	3.019 ± 0.77
Somatic Cells/ml	399 ± 395.54	1059.615 ± 2559.29	395.235 ±439.31	293.2 ± 559.62	108.563 ± 135.14
Urea mg/dl	17.43 ± 13.54	22.008 ± 7.93	22.606 ± 3.86	19.553 ± 3.36	21 ± 3.79
Acetone (-) mM/L	6.11 ± 0.34	0.444 ± 0.22	0.072 ± 0.13	0.119 ± 0.16	0.105 ±0.09
Lactose %	3.586 ± 0.99	3.983 ± 0.71	5.539 ± 0.27	5.598 ±0.73	5.843 ±0.55
Non-fat Solids %	20.86 ± 5.83	16.638 ± 3.57	10.678 ± 1.07	10.603 ± 3.08	10.105 ± 0.54
	n=16	n=13	n=17	n= 15	n=16

Effect	Num DF	Den DF	F Value	Pr>F
Time	4	72	35.12	<.0001

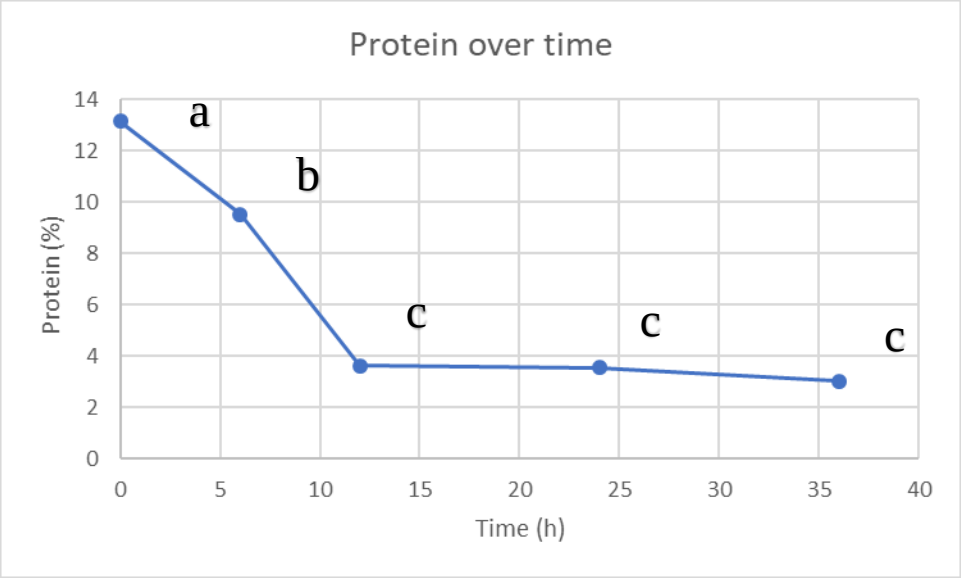

Figure 1. Change in protein over time. a, b, c- means with different letters are different (P<0.05)

Effect	Num DF	Den DF	F Value	Pr>F
Time	4	72	35.28	<.0001

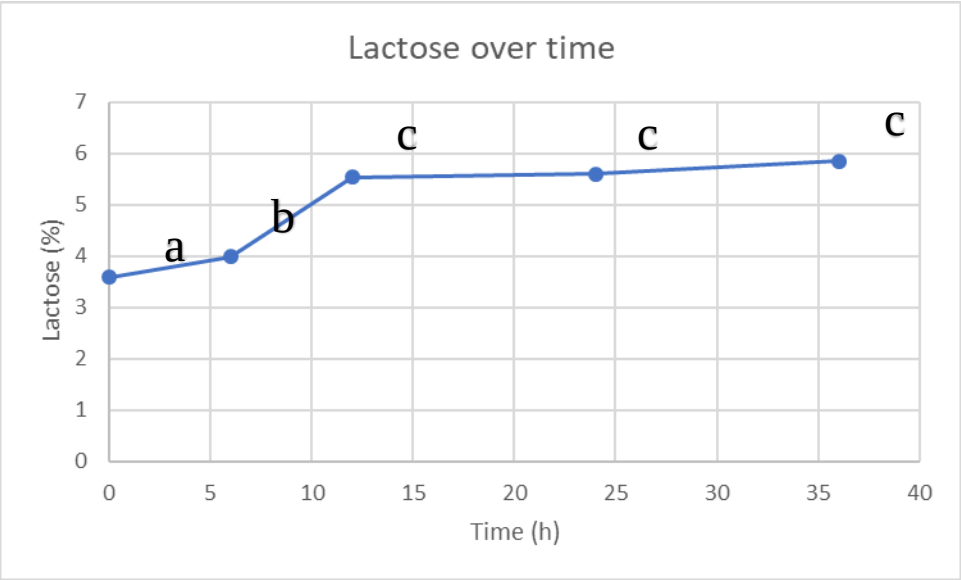

Figure 2. Change in lactose over time. a, b, c- means with different letters are different (P<0.05*)*

Effect	Num DF	Den DF	F Value	Pr>F
Time	4	72	31.61	<.0001

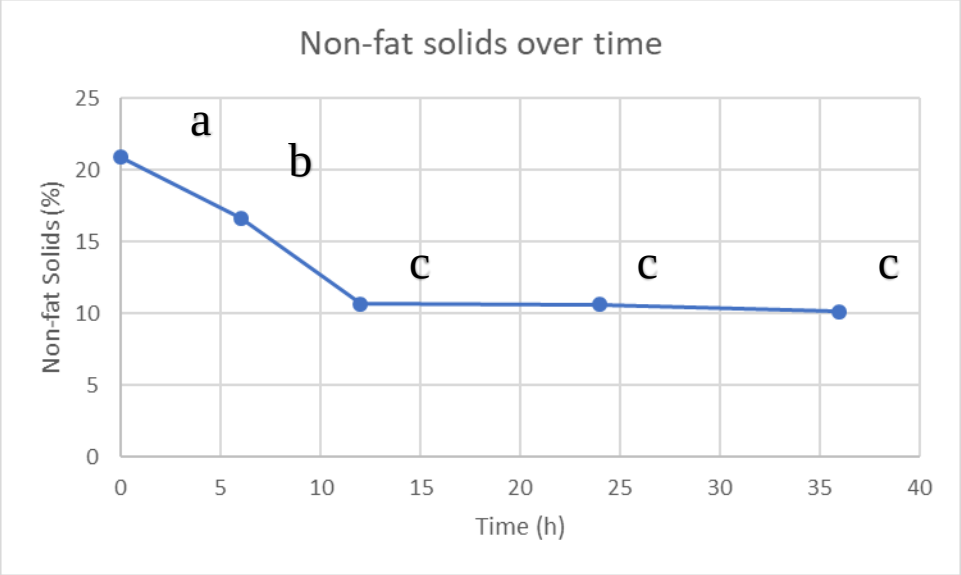

Figure 3. Change in non-fat solids over time. a, b, c- means with different letters are different (P<0.05)

On farm measurements

The Brix average was 21.9% which is on the lower end of "good", and the Colostrometer average was 1.057, which is considered "poor", however the Colostrometer does not accurately determine concentrations above 1.1, which means the data could be slightly skewed left. On farm measurement means are described in Table 2.

Table 2. Evaluation of colostrum quality by on-farm tests: Brix Refractometer and Colostrometer.

Brix	21.29% ± 9.68%
Colostrometer	1.057 ± 0.058

Immunoglobulins

The greatest immunoglobulin concentration at h 0 was IgG with 9.994 ± 5.48 mg/ml, followed by IgA with 6.735 ± 4.348 mg/ml, and IgM 2.289 ± 1.98 mg/ml. Immunoglobulin concentration means are described in Table 3.

Table 3. Immunoglobulin concentrations of colostrum collected at h 0.

	h 0
IgA mg/ml	6.735 ± 4.348
IgM mg/ml	2.289 ± 1.98
IgG mg/ml	9.994 ± 5.48

Correlations between BCS and fat measurements

Correlations between BCS and fat measurements are described in Table 4. The Body Condition Score system correlated significantly with weight (P=0.0208, R=0.48934), rump fat* (P=0.0002, R=0.70804), percent body fat (P<0.0001, R=0.77802), $17^{th}/18^{th}$ rib eye area (P=0.0226, R=0.48368), $13^{th}/14^{th}$ rib eye area (P=0.0308, R=0.46104), intramuscular fat (P=0.0221, R=0.48515) and rump fat (P<.0001, R=0.77802). BCS was also significantly correlated with the combination of $13^{th}/14^{th}$ fat, $13^{th}/14^{th}$ loin eye area and rump fat (P<0.0001, R=0.80).

Table 4. Correlations between BCS and body fat measurements.

	Weight	RumpFat*	% Body Fat	17th/18th REA	17th/18th FAT	13th/14th REA	13th/14th FAT	IMF	RumpFat
BCS	0.48934	0.70804	0.77802	0.26098	0.48368	0.46104	0.67849	0.48515	0.77802
(n=22)	0.0208	0.0002	<.0001	0.2407	0.0226	0.0308	0.0005	0.0221	<.0001

Correlations between fat measurements and colostrum composition

The body composition measurements were compared to the colostrum composition values using PROC CORR. BCS significantly correlated with lactose at 36 h (P=0.0282, R=0.54743) and colostral fat at 36 h (P=0.0412, R=0.51504) as seen in Figures 4 and 5. Percent body fat was significantly correlated with lactose at 36 h (P=0.0253, R=0.55611) which can be seen in Figure 6. Rib eye area at the 17^{th} and 18^{th} ribs was significantly correlated with lactose at 24 h (P=0.0140, R=-0.61814) which is visible in Figure 7. Fat at the $17^{th}/18^{th}$ ribs was significantly correlated with protein at 0 h (P=0.0050, R=-0.66490), lactose at 0 h (P=0.0081, R=0.63558), non-fat solids at 0 h

(P=0.0048, R=-0.66618) and colostral fat at 36 h (P=0.0154, R=0.59326) which can be observed in Figures 8, 9, 10, and 11. Fat at the $13^{th}/14^{th}$ ribs was significantly correlated with protein at 0 h (P=0.0234, R=-0.56228), colostral fat 12 h (P=0.0041, R=0.65819), protein at 24 h (P=0.0328, R=0.55222), non-fat solids at 24 h (P=0.2020, R=0.59140), and colostral fat at 36 h (P=0.0005, R=0.76836) all of which can be observed in Figures 12, 13, 14, 15 and 16. Intramuscular fat was significantly correlated with colostral fat at 12 h (P=0.0004, R=0.75630) and colostral fat at 36 h (P=0.0002, R= 0.79374) which can be seen in Figures 17 and 18. The second rump fat measurement significantly correlated with colostral fat at 12 h (P=0.0099, R=0.60634) and lactose at 36 h (P=0.0253, R=0.55611) which can be seen in Figures 19 and 20. All p-values are available in the appendix, Table A 3.

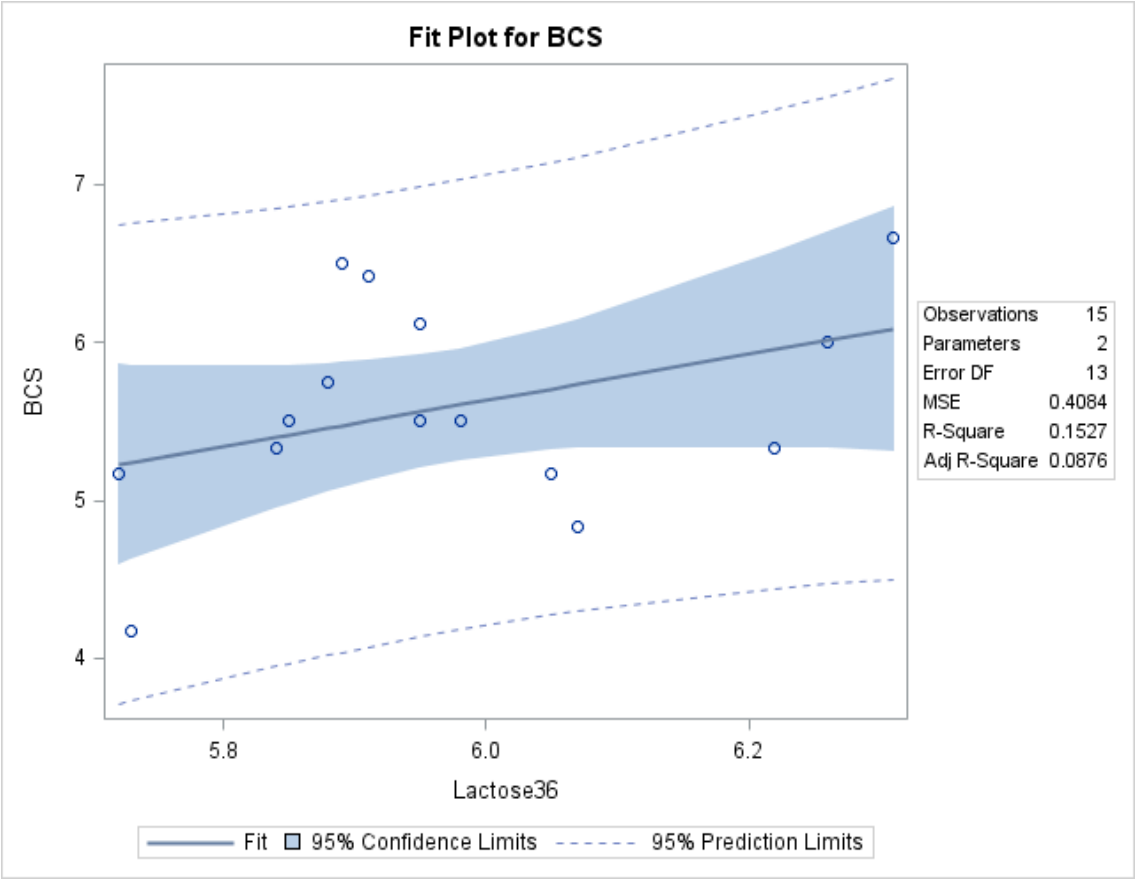

Figure 4. BCS vs lactose at h 36. (P=0.0282, R=0.54743)

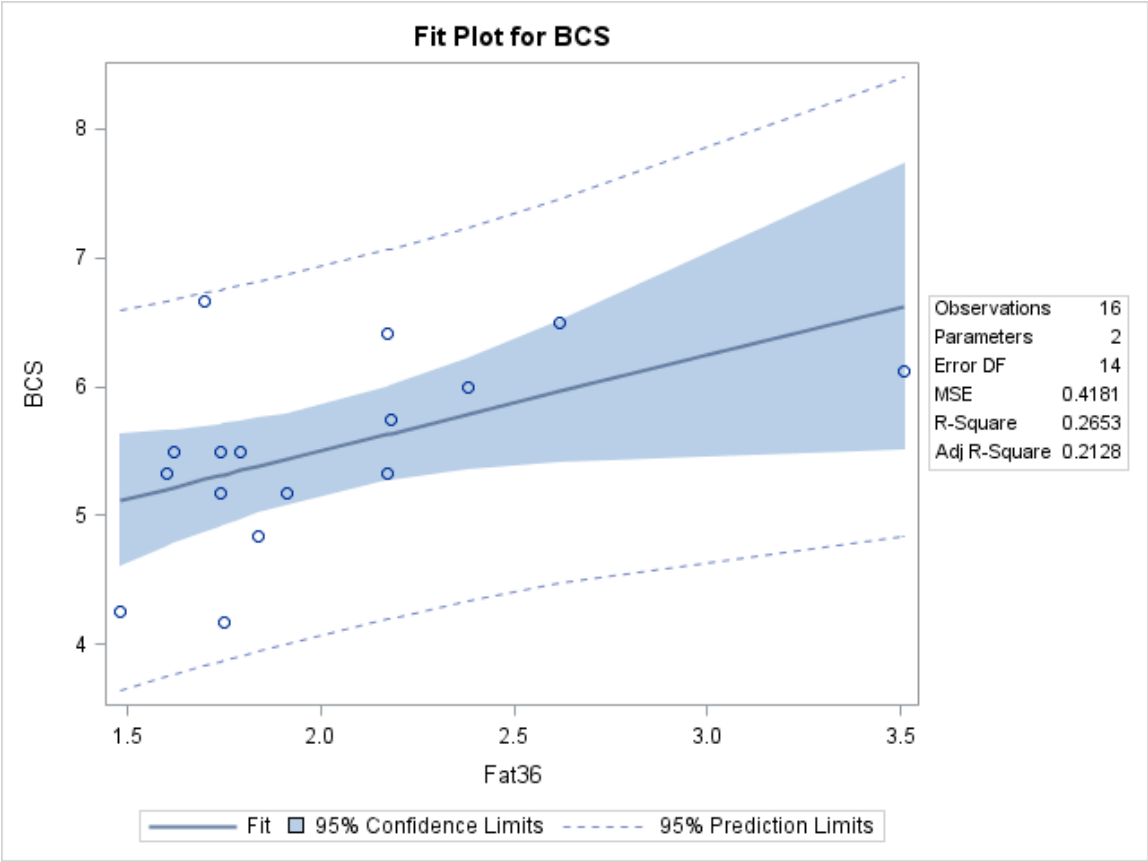

Figure 5. BCS vs colostral fat at h 36. **(**P= 0.0412, R=0.51504)

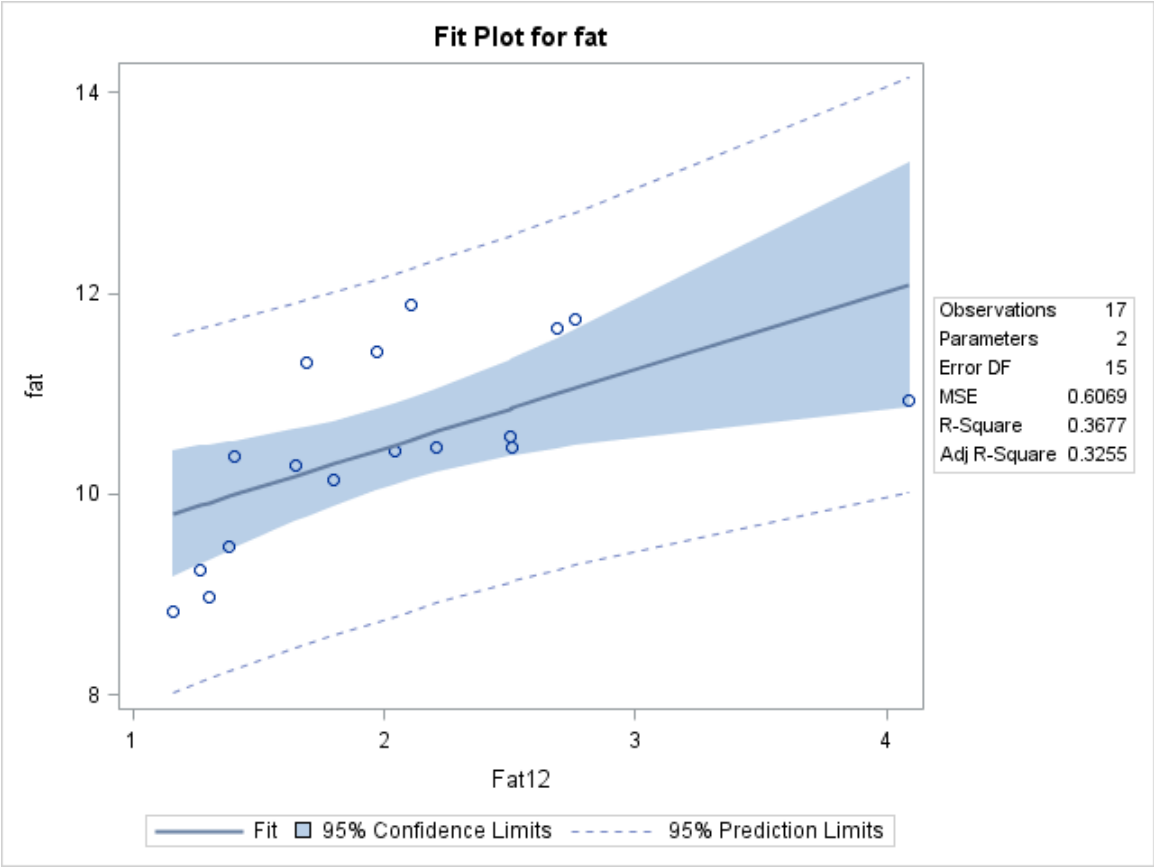

Figure 6. Percent fat vs lactose at h 36**. (**P=0.0253**,** R=0.55611)

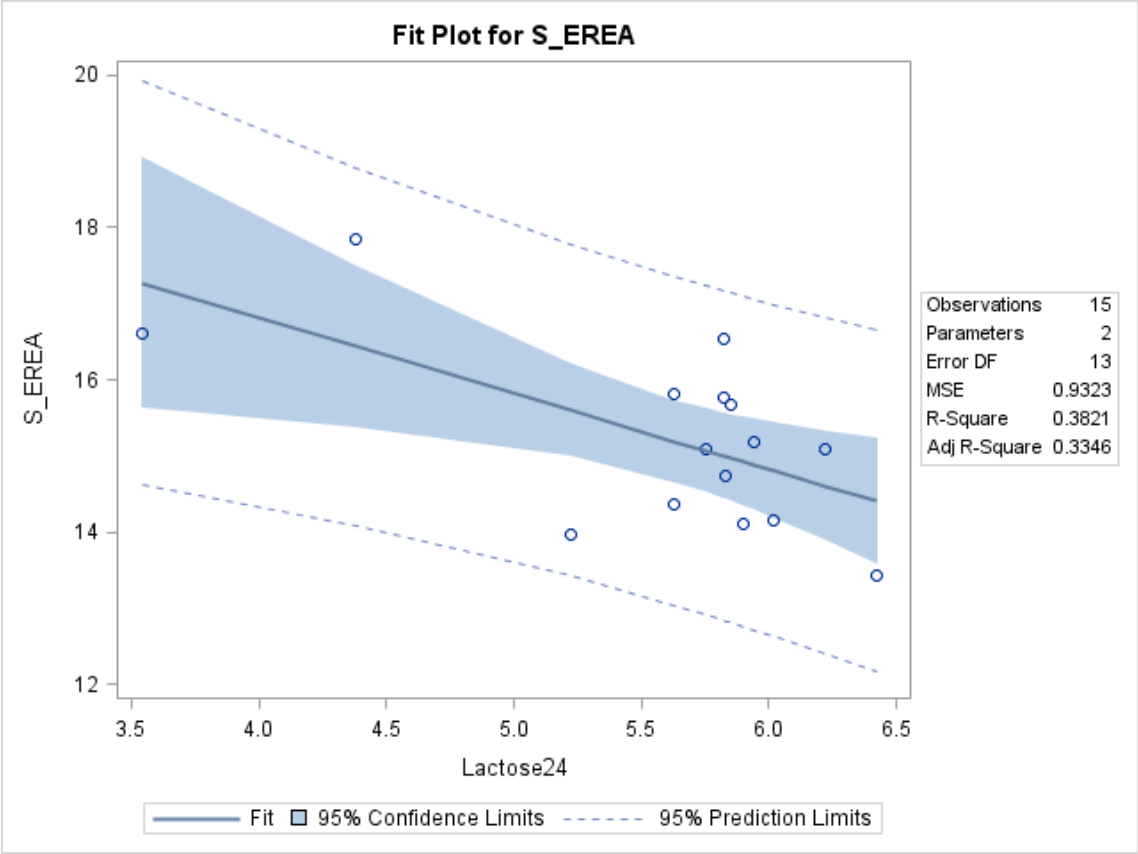

Figure 7. 17th/18th rib eye area vs lactose at h 24. (P=0.0140, R=-0.61814)

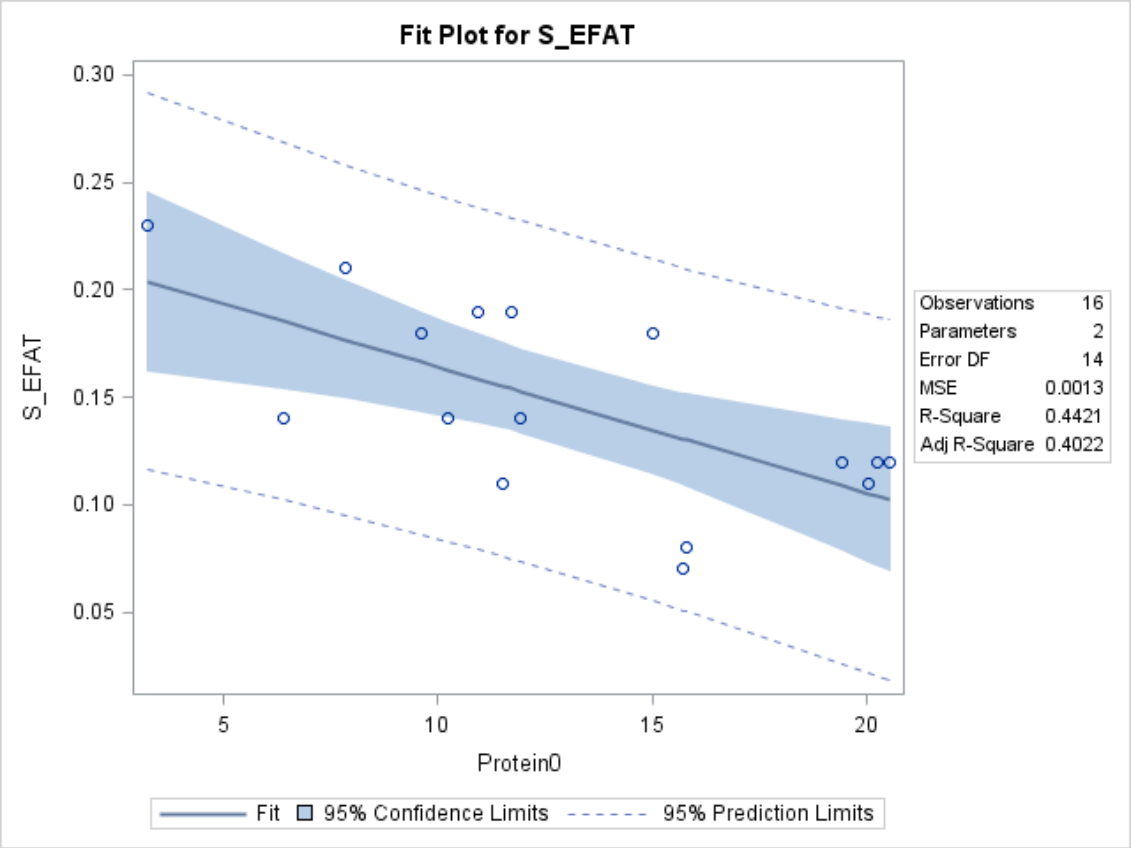

Figure 8. 17th/18th rib fat vs protein at h 0. (P=0.005, R=-0.66490)

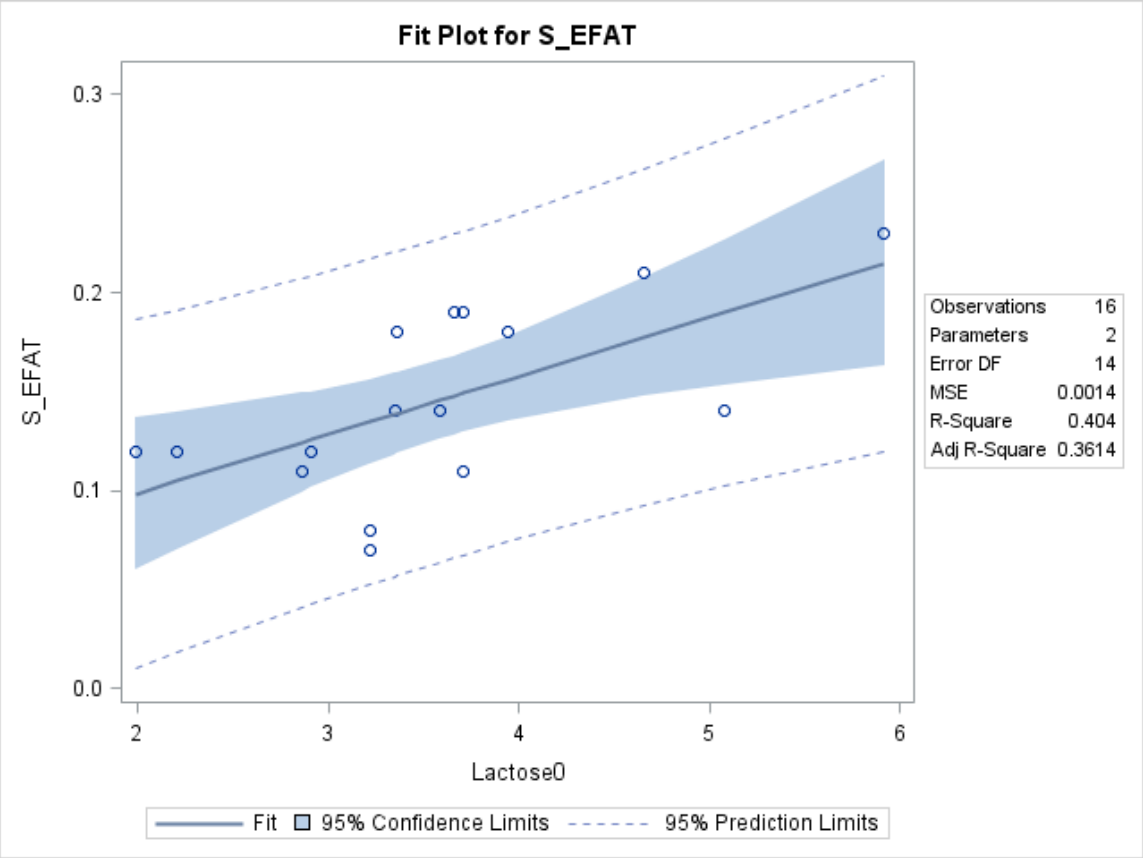

Figure 9. 17th/18th rib fat vs lactose at h 0. (P=0.0081, R=0.63558)

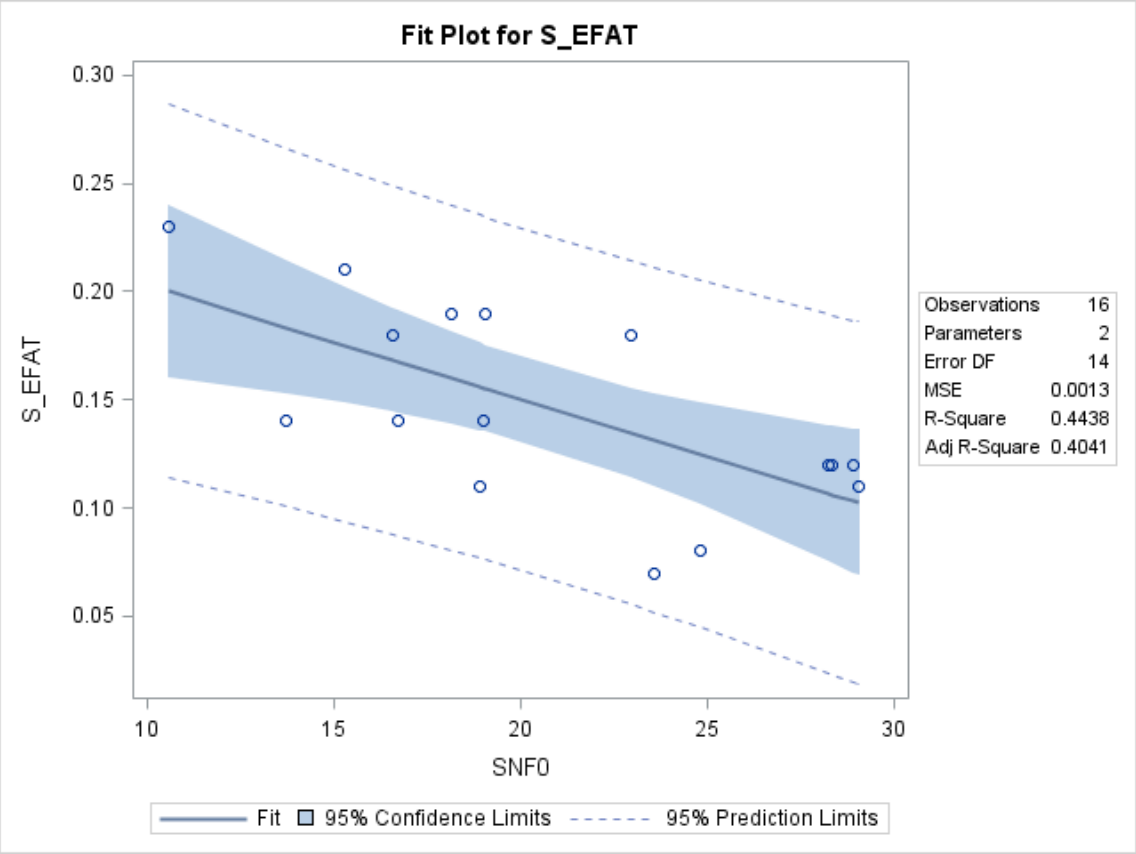

Figure 10. 17th/18th rib fat vs non-fat solids at h 0. **(**P=0.0048**,** R=-0.66618)

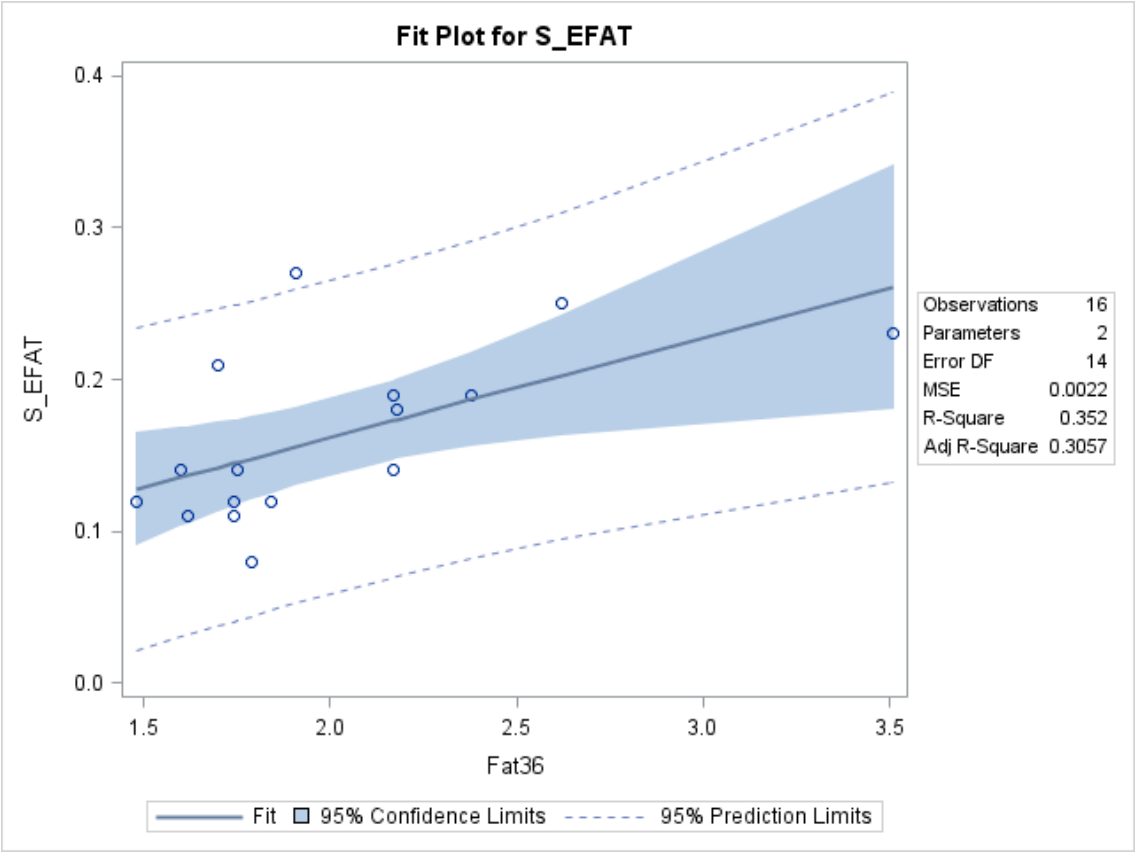

Figure 11. 17th/18th rib fat vs fat at h 36. **(**P=0.0154**,** R=0.59326)

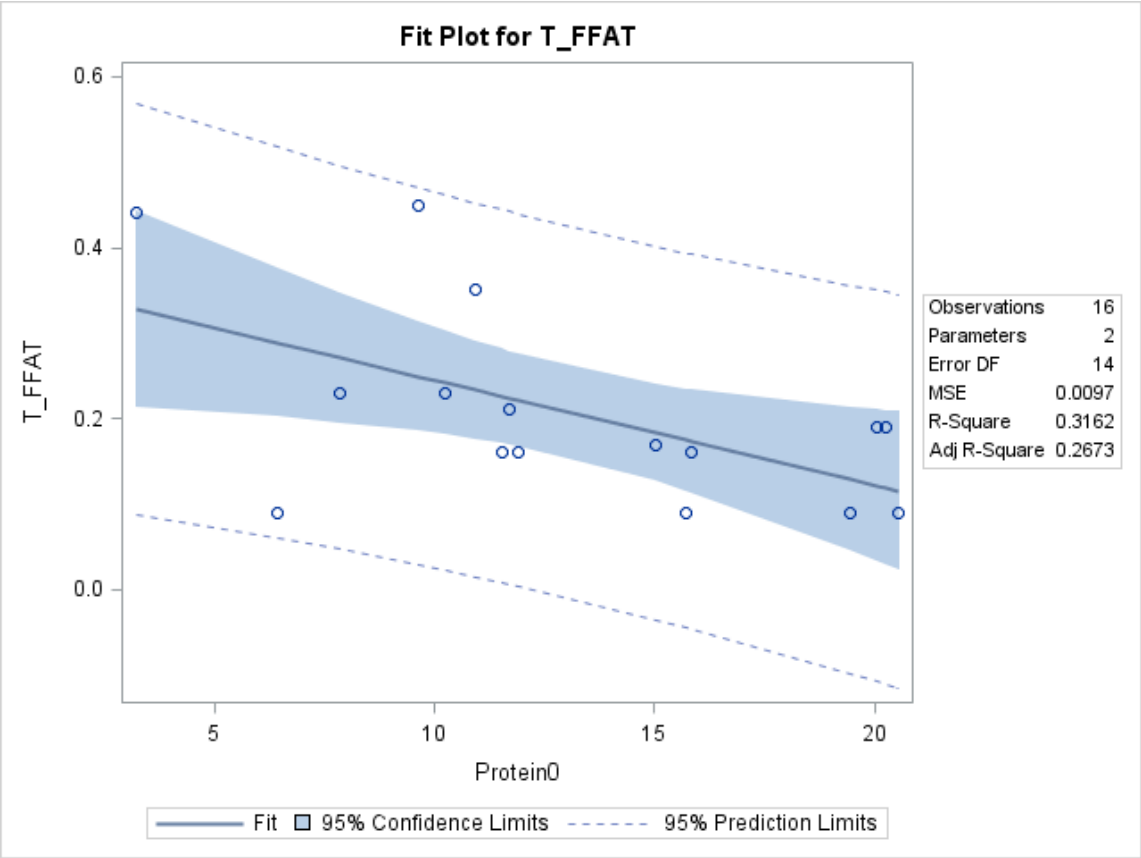

Figure 12. $13^{th}/14^{th}$ rib fat vs protein at h 0. (P=0.0234, R=-0.56228)

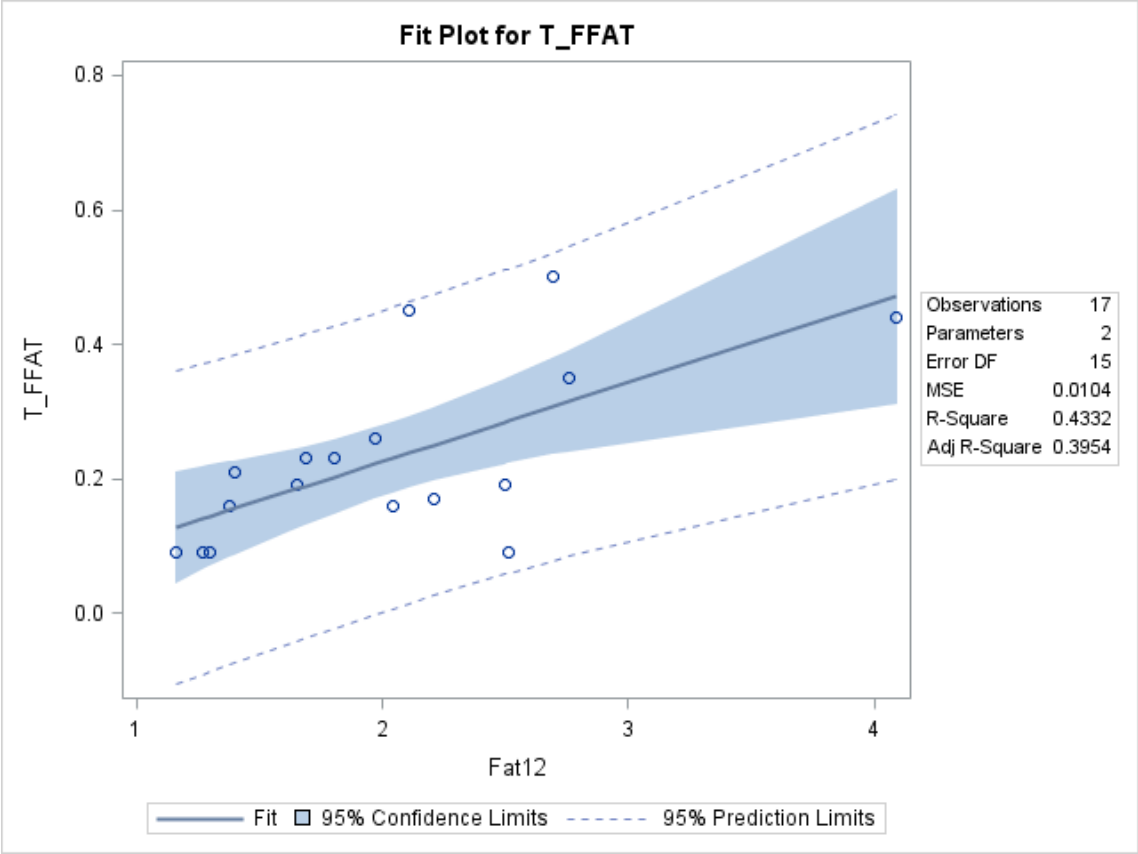

Figure 13. $13^{th}/14^{th}$ rib fat vs fat at h 12. **(**P=0.0041**,** R=0.65819)

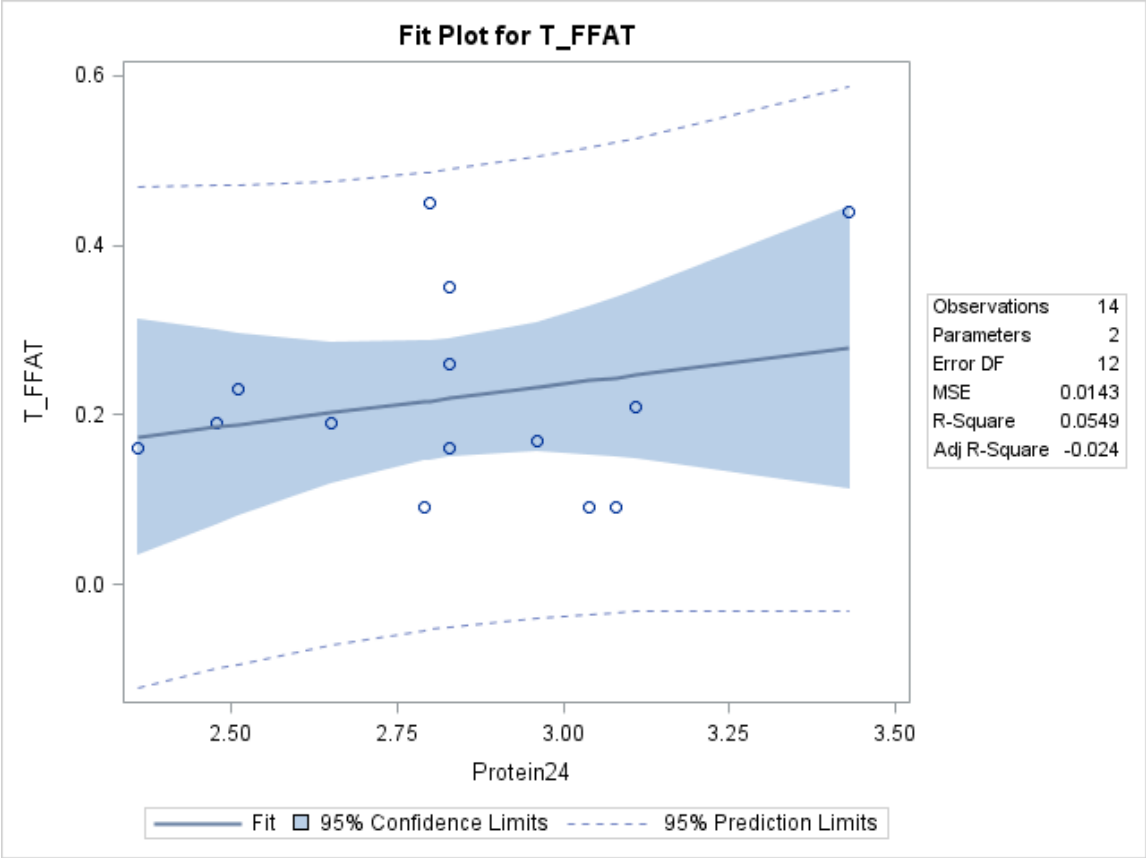

Figure 14. 13th/14th rib fat vs protein at h 24**. (**P=0.0328**,** R=0.55222)

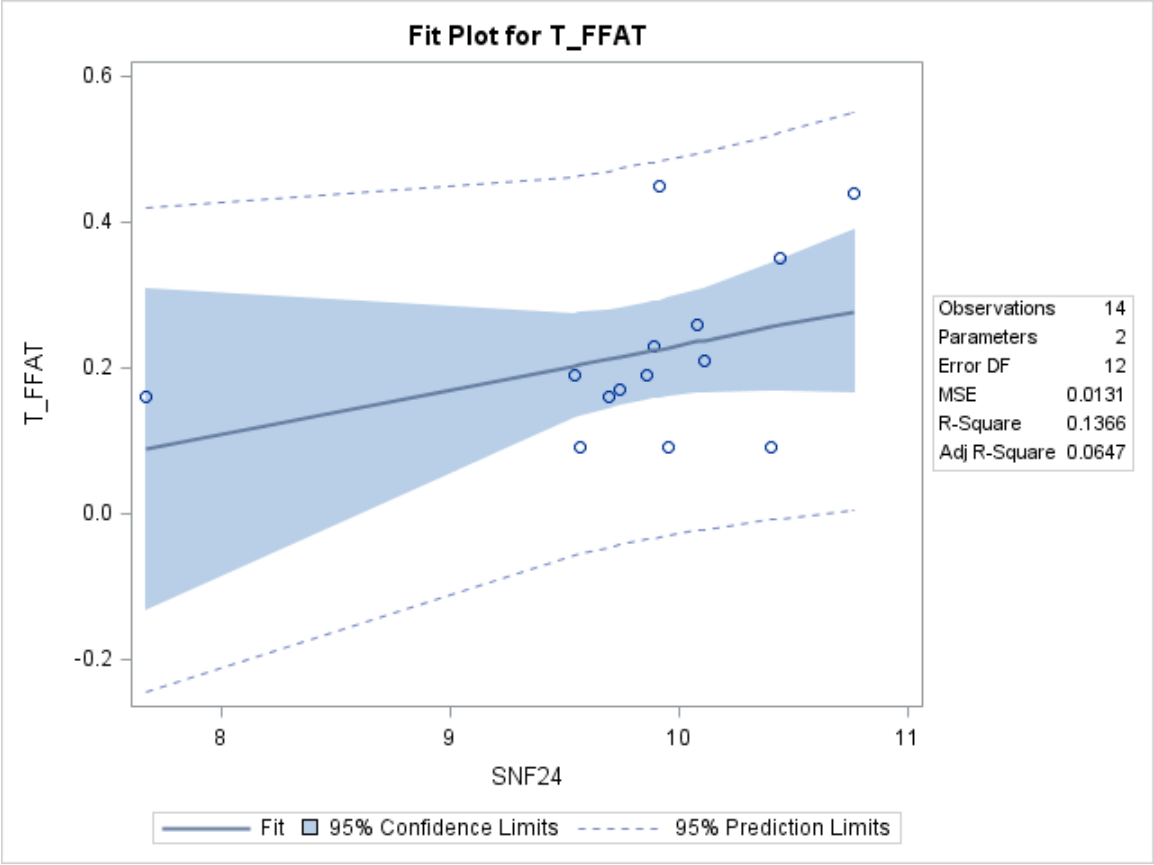

Figure 15. 13th/14th rib fat vs non-fat solids at h 24. (P=0.0202, R=0.59140)

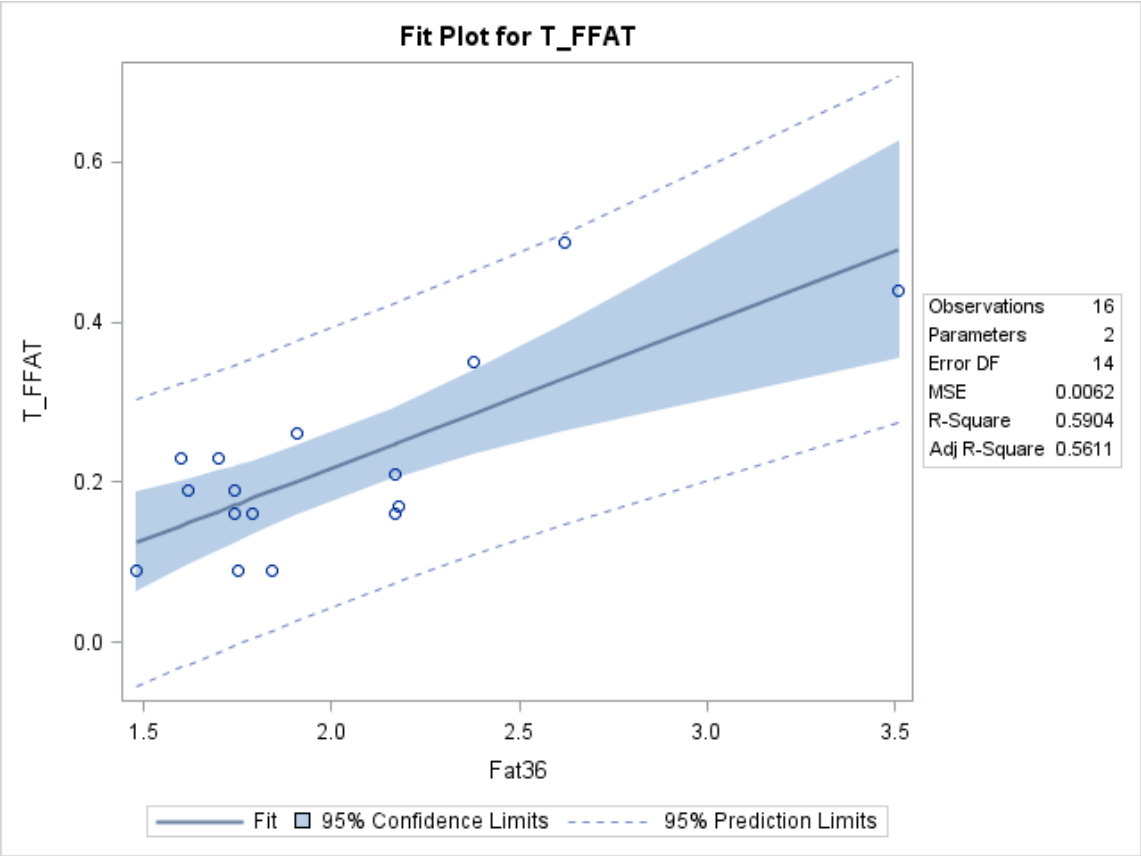

Figure 16. 13th/14th rib fat vs fat at h 36. (P=0.0005, R=0.76836)

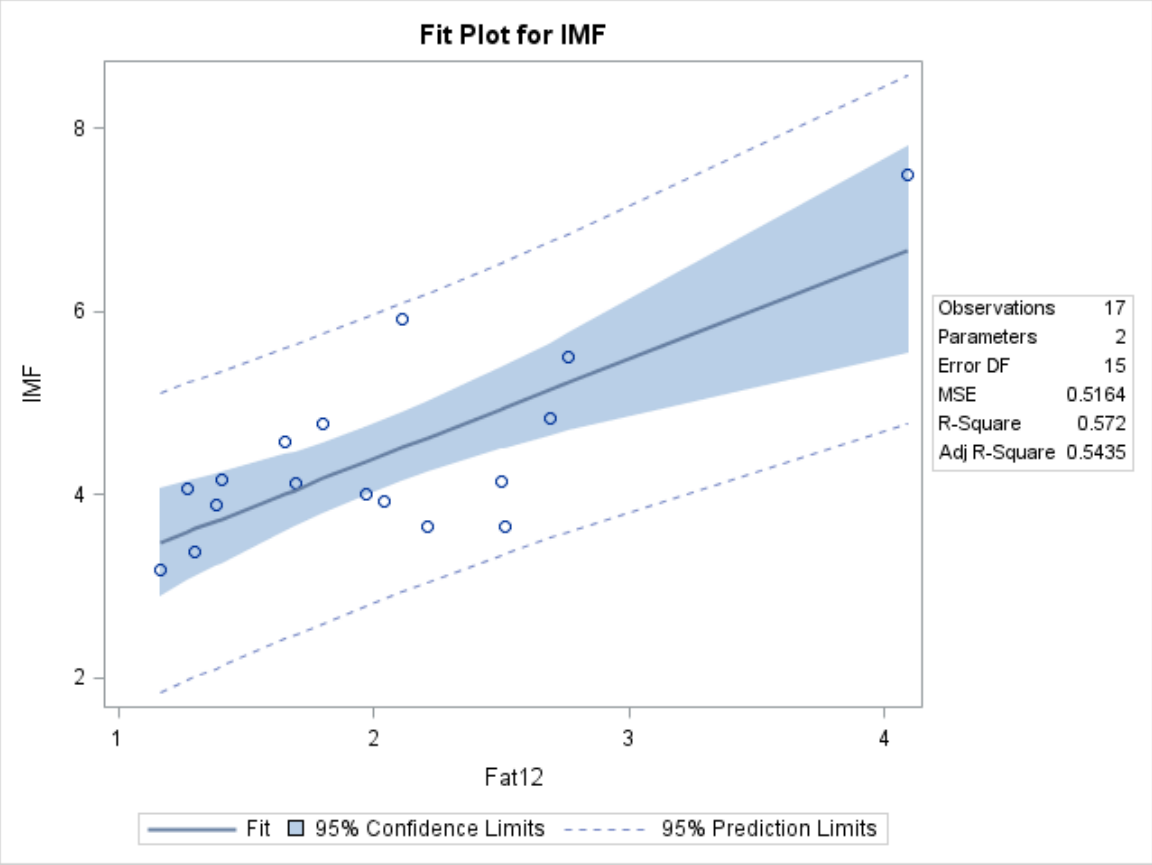

Figure 17. Intramuscular fat vs fat at h 12. (P=0.0004, R=0.75630)

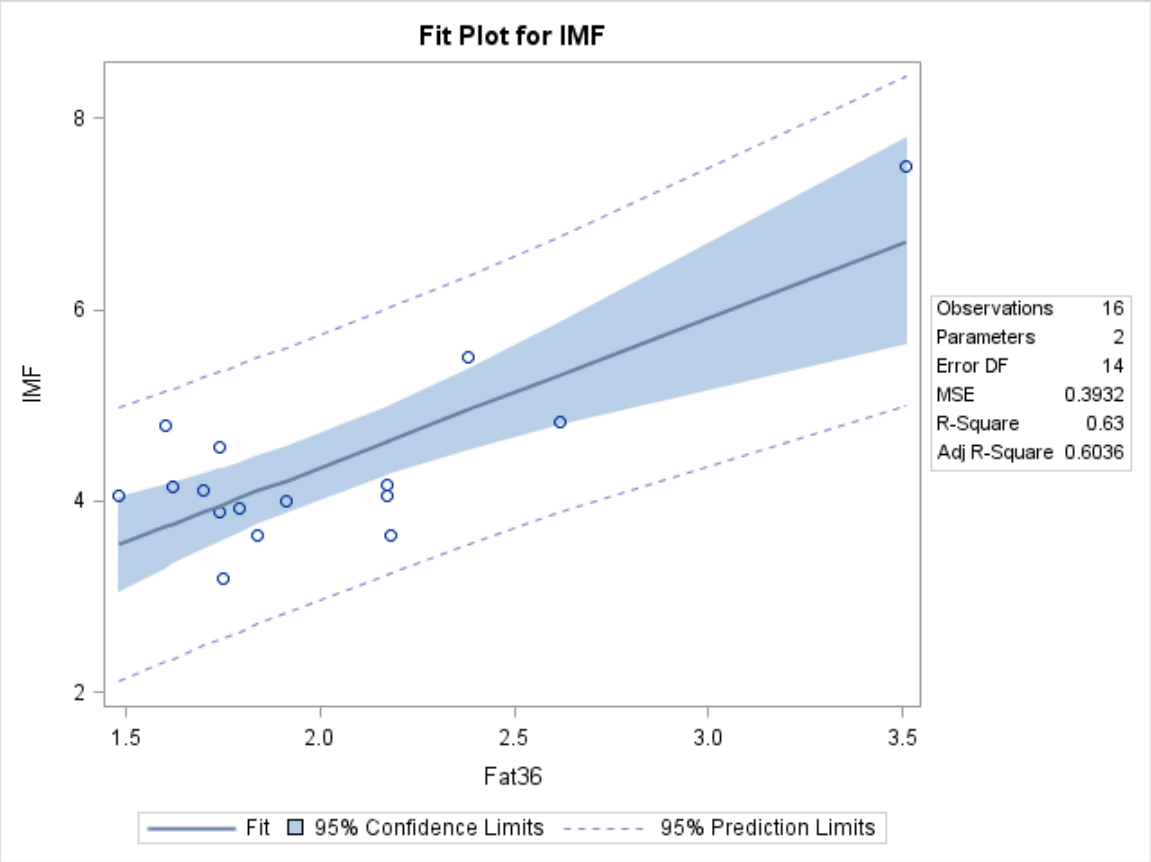

Figure 18. Intramuscular fat vs fat at h 36. (P=0.0002, R=0.79374)

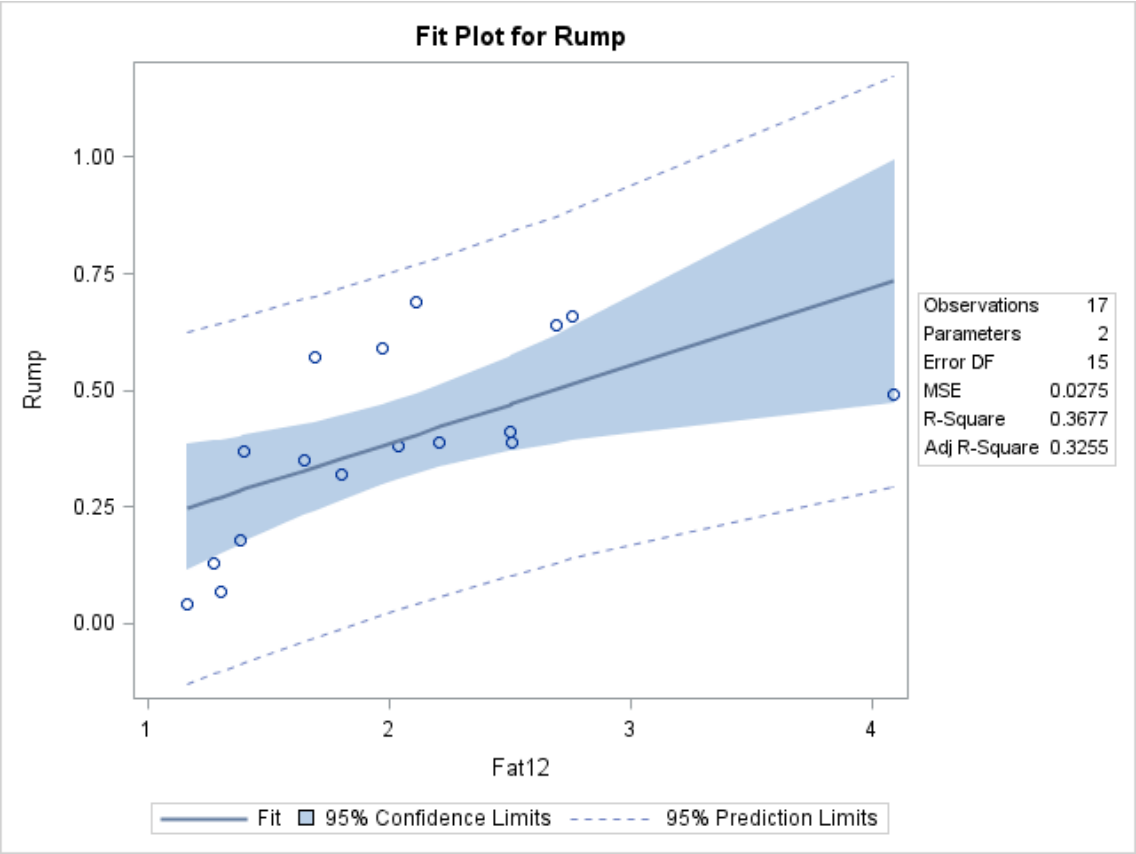

Figure 19. Rump fat vs fat at h 12. **(**P=0.0099**,** R=0.60634)

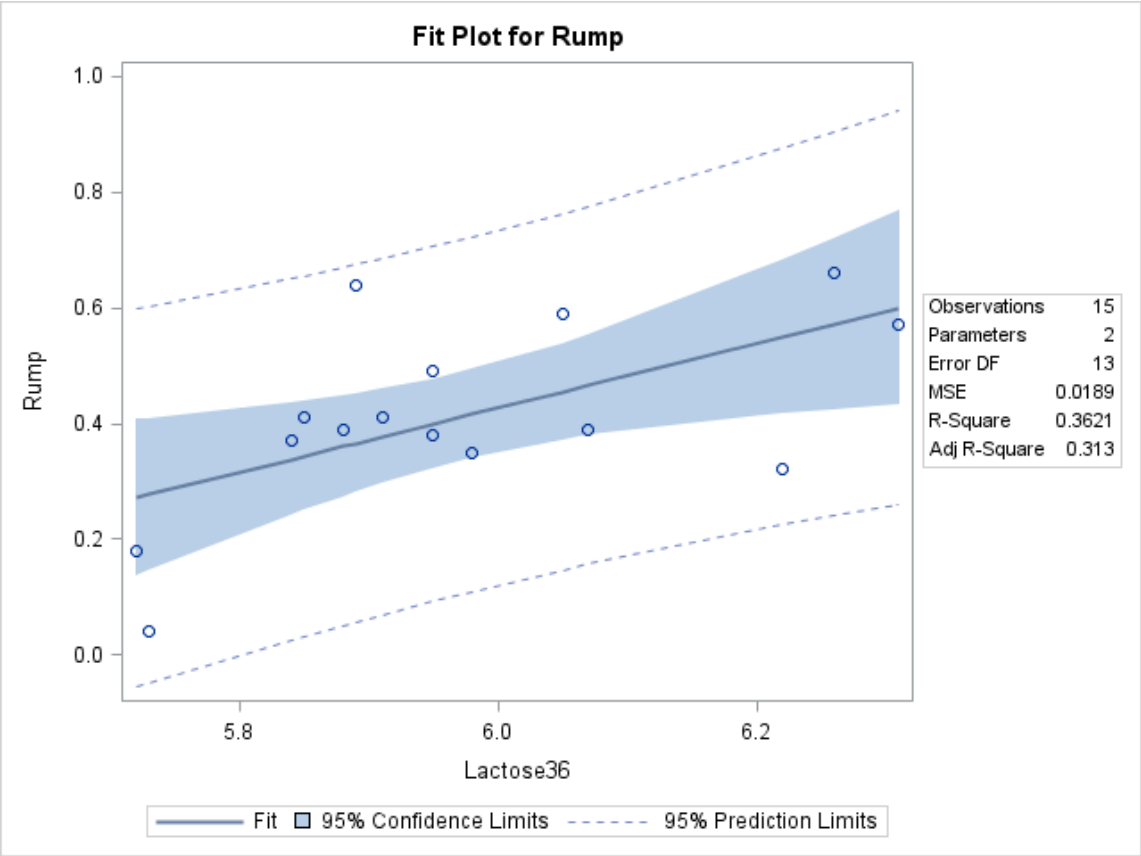

Figure 20. Rump fat vs lactose at h 36. **(**P=0.0253**,** R=0.55611)

Correlations between fat measurements and immunoglobulins

When comparing body composition measurements and immunoglobulin concentrations of colostrum taken at 0 h, the $13^{th}/14^{th}$ rib eye area showed a trend with IgG concentrations (P=0.0801, R=0.41149). No body fat measurements had a significant correlation with immunoglobulin concentrations, which can be seen in Table 5.

Table 5. Correlations between immunoglobulins and body fat measurements

	Weight	BCS	RumpFat*	% Body Fat	17th/18th REA	17th/18th FAT	13th/14th REA	13th/14th FAT	IMF	RumpFat
IgA	0.08302	-0.09409	-0.18842	0.04673	0.13230	0.28762	-0.02371	0.22409	0.8797	0.04673
(n=19)	0.7355	0.7016	0.4398	0.8493	0.5893	0.2324	0.9232	0.3564	0.7203	0.8493
IgM	0.28824	0.09905	-0.04234	0.24356	0.21803	0.30585	0.29352	0.32453	0.05667	0.24356
(n=19)	0.2314	0.6866	0.8634	0.3150	0.3699	0.2029	0.2226	0.1752	0.8178	0.3150
IgG	0.26467	-0.06637	-0.18656	0.03445	0.38097	-0.00087	0.41149	0.10720	0.01723	0.03445
(n=19)	0.2735	0.7872	0.4444	0.8886	0.1076	0.9972	0.0801	0.6622	0.9442	0.8886

Correlations between fat measurements and on farm evaluations

Body composition measurements, weight, BCS, rump fat*, percent body fat, $17^{th}/18^{th}$ rib eye area, $17^{th}/18^{th}$ fat, $13^{th}/14^{th}$ rib eye area, $13^{th}/14^{th}$ fat, intramuscular fat and rump fat had in no significant correlations with the on-farm colostrum evaluations, which can be observed in Table 6.

Table 6. Correlations between on-farm evaluations and body fat measurements.

	Weight	BCS	RumpFat*	% Body Fat	17th/18th REA	17th/18th FAT	13th/14th REA	13th/14th FAT	IMF	RumpFat
Brix	0.01196	0.19729	-0.07906	0.23162	-0.07851	0.14336	0.09533	0.19650	-0.07572	0.23162
(n=19)	0.9612	0.4182	0.7477	0.3400	0.7494	0.5582	0.6979	0.4446	0.7580	0.3400
Colostrometer	-0.07270	0.04639	0.23338	0.19691	-0.34616	-0.07787	-0.2503	-0.06719	-0.09992	0.19691
(n=19)	0.7674	0.8504	0.3363	0.4191	0.1466	0.7514	0.3013	0.7846	0.6840	0.4191

Correlations between immunoglobulins and on farm evaluations

Brix refractometry correlated significantly with IgA (P=0.0294, R=0.52787) and IgM (P=0.0024, R=0.68545) which can be seen in Table 7.

Table 7. Correlations between immunoglobulins and on-farm evaluations.

	Brix	Colostrometer
IgA	0.52787	0.09995
(n=17)	0.0294	0.7027
IgM	0.68545	0.13868
(n=17)	0.0024	0.5956
IgG	0.27447	0.02392
(n=17)	0.2864	0.9274

CHAPTER IV

DISCUSSION AND CONCLUSIONS

Discussion

The composition measurements of colostrum and mature milk were compared to data from previous research. Percent fat, protein, lactose and somatic cell count were similar to data collected in research which evaluated the differences in composition from hand milked mares and machine milked mares (Caroprese et al., 2007). Similarly, percent fat was equal to that found in a 1995 study of mare milk composition (Csapó et al., 1995). Again colostrum collected from this group of mares resulted in similar fat, protein, lactose and non-fat solid concentrations as mares fed a starch and sugar concentrate (Hoffman et al., 1998).

The significant change in colostrum composition over time ($P<0.001$) is similar to previous data (Rouse and Ingram, 1970; Hoffman et al., 1998). However, the swiftness of this change was more pronounced during the current study when compared to the aforementioned data, with the majority of compositional changes occurring within the first 12 h post-partum. Again, when compared to studies that evaluated changes over the first 45 d, or 150 d, data collected over the 36 h post parturition in the current study displayed the majority of the changes from colostrum to occur (Gibbs et al., 1982; Csapó et al., 1995).

Immunoglobulin concentrations in this study, IgG=9.994 ±5.48, IgA=6.735±4.348, IgA=2.289±1.98 mg/ml, were lower than those found in a 1970 study using where IgG and IgT were measured at 45 and 15 mg/ml, and a study of quarter horse mares in 2000, which found IgGa, IgGb, IgGc, IgG(T) and IgA concentrations to be 82, 183, 0.3, 44, 9 mg/ml (Rouse and Ingram, 1970; Sheoran et al., 2000). The values are also lower than immunoglobulin concentrations, IgG- 90g/l, IgA- 9.51 g/l, and greater than concentrations of IgM- 1.52 g/l, found in pre-suckle colostrum in a previous study (Hoffman et al., 1999).

Evaluation of colostrum immediately after parturition using Brix Refractometry resulted in 21.29±9.68% total dissolved solids. This would be considered low-quality if using an early sugar refractometer, which was determined to qualify colostrum as "good-quality" if it resulted with 23% or higher (Chavatte et al., 1998). That being said, a prior study determined that use of a colostrometer was a more effective method of evaluating colostrum quality post parturition (Waelchli et al., 1990). In contrast, a review written by McCue (2014) stated that both the Brix Refractometer and Colostrometer were viable options of colostrum, and relative to the current study would consider the colostrum collected in this study "good" per Brix % and "poor" per specific gravity of the Colostrometer. The Brix data was lower than that of a previous study of hay fed and concentrate supplemented mares but consistent with the specific gravity of the same colostrum (Coverdale et al., 2014)

Body condition score was significantly correlated with weight, rump fat*, % body fat, fat at the $17^{th}/18^{th}$ ribs, rib eye area at the $13^{th}/14^{th}$ ribs, at at the $13^{th}/14^{th}$ ribs,

intramuscular fat and rump fat. This is consistent with past research that validated use of the BCS system and further validates its continued use in the equine industry (Henneke et al., 1983; Carter et al., 2008). This also further substantiates the use of ultrasonic fat measurements in research settings, building upon past research that verified its use (Gentry et al., 2004; Silva et al., 2015). Further investigation of ultrasonic fat measurements should include other pockets of adipose tissue evaluated with the BCS system, such as the tail head and the "crestiness" of the neck.

The correlations between body fat measurements and colostrum composition show that mares with more fat had lower protein percentages and greater lactose and fat percentages in their colostrum. This is consistent with past research, such as Davison's work at Texas A&M in 1991, which found that mares fed excess dietary fat produced more fat in their colostrum and milk (Davison et al., 1991). When it comes to the foal, high fat content food can result in greater daily weight gain. Rapidly growing horses will deposit more bone and muscle and therefore require higher quantities of calcium, phosphorus, and lysine (Harris, 2003). One potential issue that can arise from an inappropriate diet is developmental orthopedic disease (DOD). Foal health is important for future performance success and an increase in lameness is a drain on the owners' checkbooks. To maintain appropriate calcium to phosphorus ratios, concentrate feed would need to be accessible to the foal. Furthermore, immunoglobulins make up a large portion of protein, lower amounts of protein could result in lower immunoglobulins in colostrum, although that was not proven in this study. The data suggests that overfeeding mares can be detrimental to the quality of their colostrum and milk, and therefore

detrimental to their offspring. This, combined with the other weight related issues such as metabolic disorders, laminitis, and the potential economic strain, leads to the recommendation that broodmares should be kept in within the healthy BCS range of 5-6.

There were no significant correlations between body fat measurements and immunoglobulin concentrations. No correlation was found between body fat measurements and on-farm colostrum evaluations. This makes sense considering body fat measurements had little effect on immunoglobulin concentration and immunoglobulins are historically highly correlated with Brix Refractometry and Colostrometry. This is in contrast to a study in which it was reported that overfed mares produced less IgG in their colostrum (Coverdale et al., 2014).

In the current study, Brix refractometry was significantly correlated with IgA and IgM and not with IgG which is in direct contrast with previous research, such as one study which found IgG by TIA to correlated significantly ($r=0.63$) (Quigley et al., 2013). These data are also in contrast to a study in which a sugar refractometer was used to evaluate colostrum and was found to correlate highly with IgG concentrations ($p<0.0001$, $R=0.85$) (Chavatte et al., 1998). Similarly, the data collected from the current study differed from that of another study in which specific gravity was significantly correlated ($r=0.82$) with IgG concentrations (LeBlanc et al., 1986). Variation in the technicians collecting the samples, and slight variation in the timing of the sample collections may partially explain the disparity between these data and that of previous research.

Conclusions

In conclusion, this study further validates the use of the Body Condition Score system in the equine industry as well as the use of ultrasonic fat measurements in equine research. However, the data collected suggest that the use of Brix Refractometry and the Colostrometer may not be an accurate estimate of immunoglobulin concentrations as previous thought. Also, Westervelt's equation may be less accurate than the combination of fat measurements selected in this study, which requires further evaluation. Furthermore, the data show that the significant changes in mammary secretions from colostrum to milk occur within the first 12 hours of lactation, which means that the foal has less opportunity to consume colostrum than previously thought. Finally, fatter mares produce a lower percentage of protein and higher percentages of fat and lactose which can be detrimental to their offspring.

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APPENDIX

Figure A 1. Body Condition Score System, as described by Henneke et al., (1983)

.

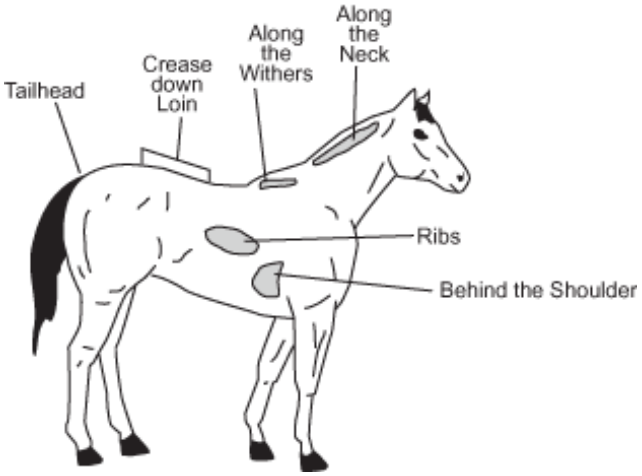


Figure A 2. Rump Fat Measurement Location

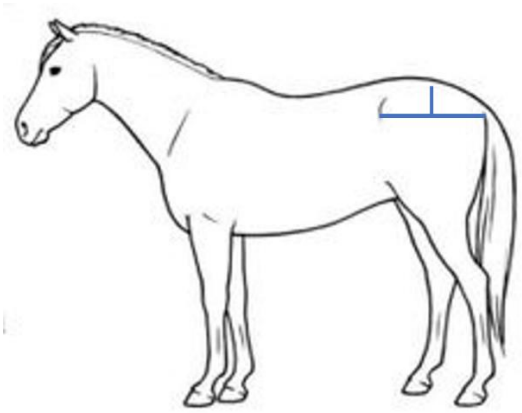
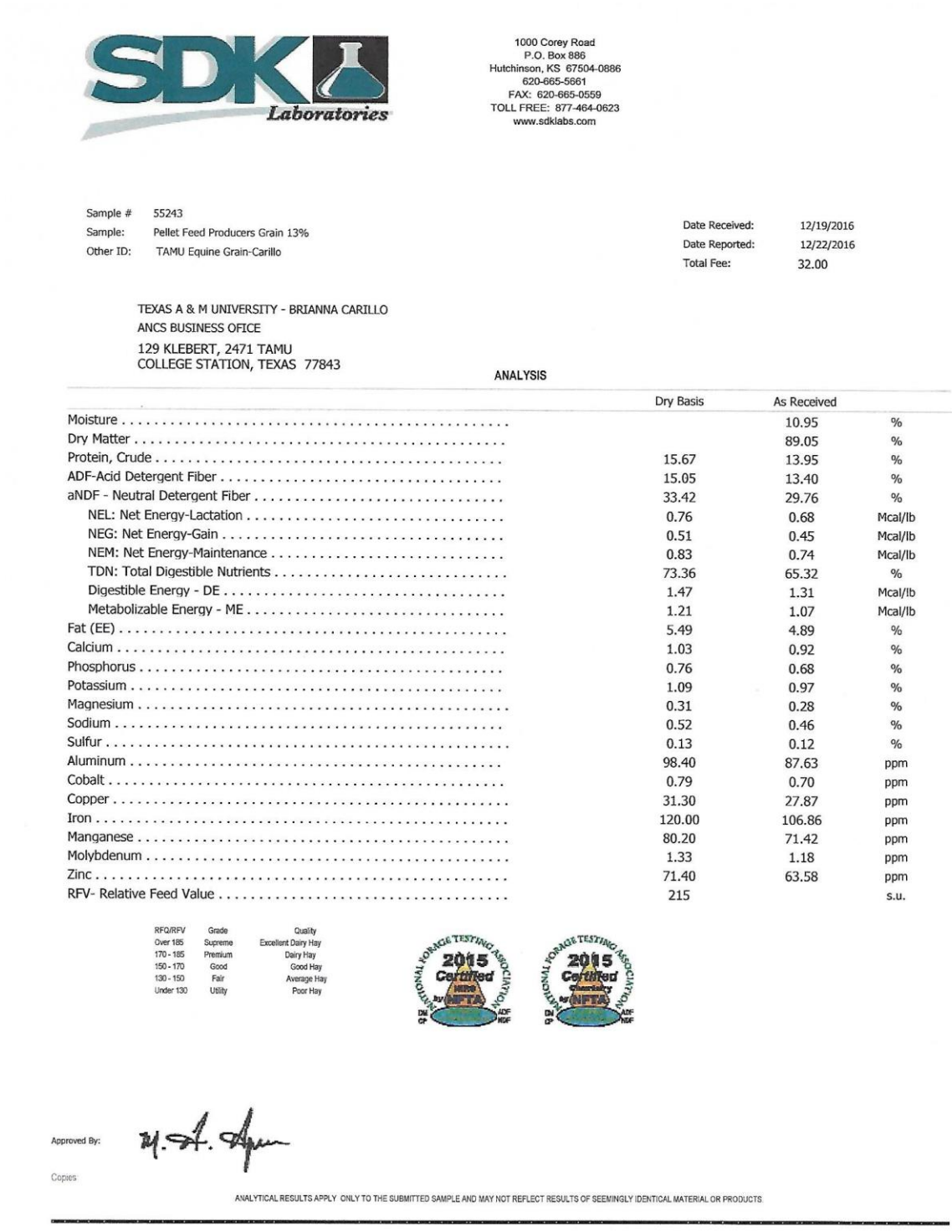


Figure A 3. Grain analysis



SDK Laboratories

1000 Corey Road
P.O. Box 886
Hutchinson, KS 67504-0886
620-665-5661
FAX: 620-665-0559
TOLL FREE: 877-464-0623
www.sdklabs.com

Sample # 55243
Sample: Pellet Feed Producers Grain 13%
Other ID: TAMU Equine Grain-Carillo

Date Received: 12/19/2016
Date Reported: 12/22/2016
Total Fee: 32.00

TEXAS A & M UNIVERSITY - BRIANNA CARILLO
ANCS BUSINESS OFICE
129 KLEBERT, 2471 TAMU
COLLEGE STATION, TEXAS 77843

ANALYSIS

	Dry Basis	As Received	
Moisture		10.95	%
Dry Matter		89.05	%
Protein, Crude	15.67	13.95	%
ADF-Acid Detergent Fiber	15.05	13.40	%
aNDF - Neutral Detergent Fiber	33.42	29.76	%
NEL: Net Energy-Lactation	0.76	0.68	Mcal/lb
NEG: Net Energy-Gain	0.51	0.45	Mcal/lb
NEM: Net Energy-Maintenance	0.83	0.74	Mcal/lb
TDN: Total Digestible Nutrients	73.36	65.32	%
Digestible Energy - DE	1.47	1.31	Mcal/lb
Metabolizable Energy - ME	1.21	1.07	Mcal/lb
Fat (EE)	5.49	4.89	%
Calcium	1.03	0.92	%
Phosphorus	0.76	0.68	%
Potassium	1.09	0.97	%
Magnesium	0.31	0.28	%
Sodium	0.52	0.46	%
Sulfur	0.13	0.12	%
Aluminum	98.40	87.63	ppm
Cobalt	0.79	0.70	ppm
Copper	31.30	27.87	ppm
Iron	120.00	106.86	ppm
Manganese	80.20	71.42	ppm
Molybdenum	1.33	1.18	ppm
Zinc	71.40	63.58	ppm
RFV- Relative Feed Value	215		s.u.

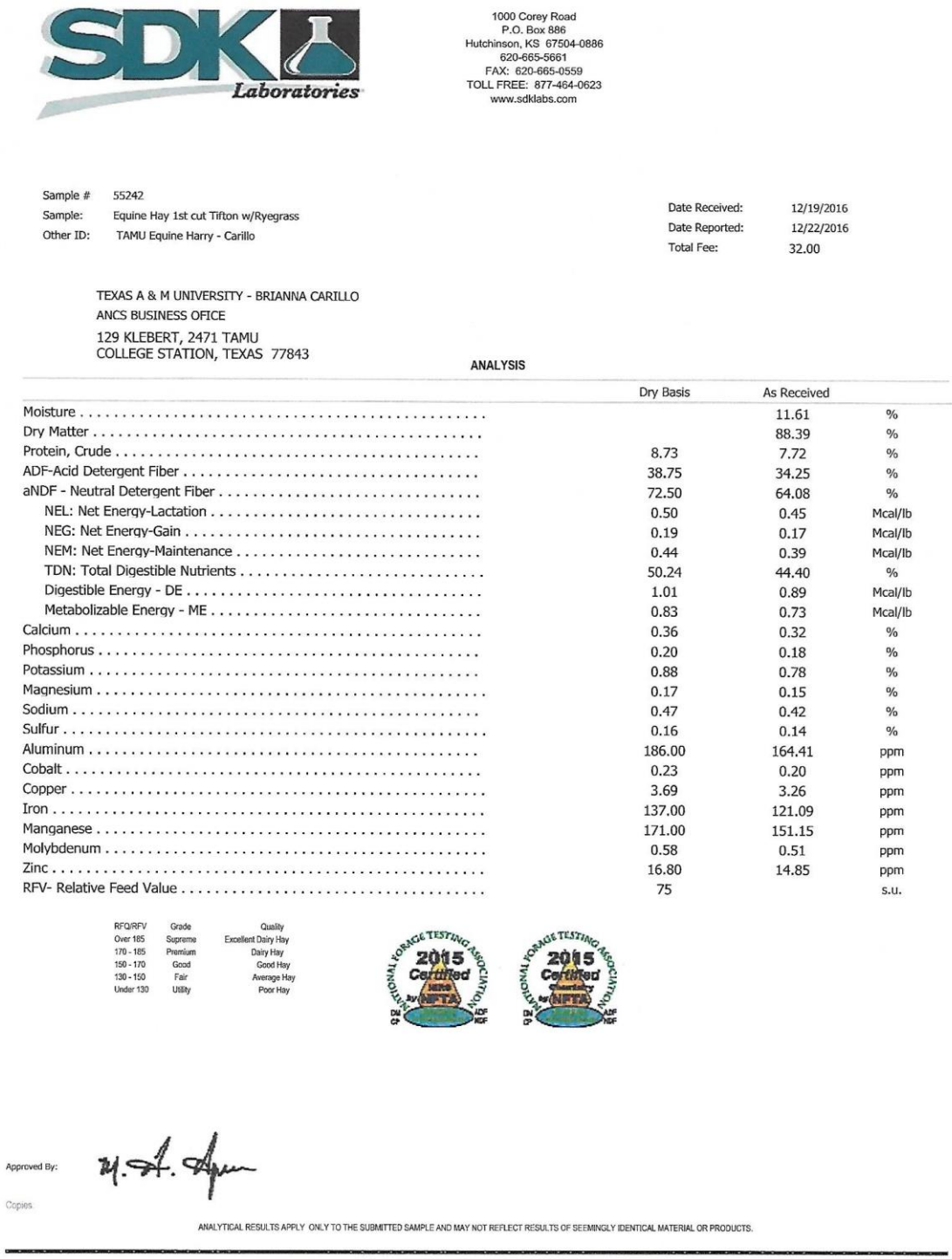
RFQ/RFV	Grade	Quality
Over 185	Supreme	Excellent Dairy Hay
170 - 185	Premium	Dairy Hay
150 - 170	Good	Good Hay
130 - 150	Fair	Average Hay
Under 130	Utility	Poor Hay

Approved By:

Copies

ANALYTICAL RESULTS APPLY ONLY TO THE SUBMITTED SAMPLE AND MAY NOT REFLECT RESULTS OF SEEMINGLY IDENTICAL MATERIAL OR PRODUCTS

Figure A 4. Hay analysis



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FAX: 620-665-0559
TOLL FREE: 877-464-0623
www.sdklabs.com

Sample # 55242
Sample: Equine Hay 1st cut Tifton w/Ryegrass
Other ID: TAMU Equine Harry - Carillo

Date Received: 12/19/2016
Date Reported: 12/22/2016
Total Fee: 32.00

TEXAS A & M UNIVERSITY - BRIANNA CARILLO
ANCS BUSINESS OFICE
129 KLEBERT, 2471 TAMU
COLLEGE STATION, TEXAS 77843

ANALYSIS

	Dry Basis	As Received	
Moisture		11.61	%
Dry Matter		88.39	%
Protein, Crude	8.73	7.72	%
ADF-Acid Detergent Fiber	38.75	34.25	%
aNDF - Neutral Detergent Fiber	72.50	64.08	%
NEL: Net Energy-Lactation	0.50	0.45	Mcal/lb
NEG: Net Energy-Gain	0.19	0.17	Mcal/lb
NEM: Net Energy-Maintenance	0.44	0.39	Mcal/lb
TDN: Total Digestible Nutrients	50.24	44.40	%
Digestible Energy - DE	1.01	0.89	Mcal/lb
Metabolizable Energy - ME	0.83	0.73	Mcal/lb
Calcium	0.36	0.32	%
Phosphorus	0.20	0.18	%
Potassium	0.88	0.78	%
Magnesium	0.17	0.15	%
Sodium	0.47	0.42	%
Sulfur	0.16	0.14	%
Aluminum	186.00	164.41	ppm
Cobalt	0.23	0.20	ppm
Copper	3.69	3.26	ppm
Iron	137.00	121.09	ppm
Manganese	171.00	151.15	ppm
Molybdenum	0.58	0.51	ppm
Zinc	16.80	14.85	ppm
RFV- Relative Feed Value	75		s.u.

RFQ/RFV	Grade	Quality
Over 185	Supreme	Excellent Dairy Hay
170 - 185	Premium	Dairy Hay
150 - 170	Good	Good Hay
130 - 150	Fair	Average Hay
Under 130	Utility	Poor Hay

Approved By:

Copies

ANALYTICAL RESULTS APPLY ONLY TO THE SUBMITTED SAMPLE AND MAY NOT REFLECT RESULTS OF SEEMINGLY IDENTICAL MATERIAL OR PRODUCTS.

Figure A 5. P-value chart

	Weight	BCS	RumpFat*	% Body Fat	17th/18th REA	17th/18th FAT	13th/14th REA	13th/14th FAT	IMF	RumpFat
Fat 0	-0.07578	-0.04345	-0.16939	0.00881	0.35322	0.08030	0.00030	0.8831	0.32893	0.00881
	0.7803	0.8730	0.5306	0.9742	0.1796	0.7675	0.991	0.7450	0.2135	0.9742
Protein 0	0.11986	-0.37871	-0.24129	-0.22239	0.01445	***-0.66490***	0.19219	***-0.56228***	-0.48740	-0.22239
	0.6584	0.1480	0.3680	0.4078	0.9576	***0.0050***	0.4758	***0.0234***	0.0555	0.4078
SCC 0	-0.07014	0.04745	0.18157	0.10203	-0.10042	0.07342	-0.12301	0.11122	-0.06280	0.10203
	0.7963	0.8615	0.5009	0.7069	0.7114	0.7870	0.6499	0.6817	0.8173	0.7069
Urea 0	-0.28407	-0.33277	-0.48751	-0.18458	-0.27976	-0.12740	-0.05357	0.09513	0.09102	-0.18458
	0.2863	0.2079	0.0554	0.4937	0.2940	0.6382	0.8438	0.7260	0.7374	0.4937
Acetone 0	-0.00261	0.33400	0.26405	0.10399	0.13062	***0.49772***	***-0.16127***	0.31327	0.28449	0.10399
	0.9923	0.2061	0.3230	0.7015	0.6297	***0.0498***	***0.0498***	0.2375	0.2856	0.7105
Lactose 0	-0.19149	0.27609	0.17538	0.12862	-0.03838	***0.63558***	-0.32084	0.35087	0.45652	0.12862
	0.4774	0.3006	0.5159	0.6350	0.8878	***0.0081***	0.2257	0.1827	0.0755	0.6350
NFS 0	0.10298	-0.38933	-0.24659	-0.23070	-0.00064	***-0.66618***	0.16124	0.48251	-0.48431	-0.23070
	0.7043	0.1361	0.3572	0.3900	0.9981	***0.0048***	0.5508	0.0584	0.0573	0.3900
Fat 6	0.20809	-0.12540	-0.29333	-0.23600	0.21746	-0.19070	0.35387	-017877	-0.01761	-0.23600
	0.4951	0.6831	0.3307	0.4376	0.4754	0.5326	0.2355	0.5590	0.9545	0.4376
Protein 6	-0.49477	-0.42605	-0.41682	-0.23909	-0.30770	-0.31486	-0.32896	-0.33271	-0.03641	-0.23909
	0.0856	0.1466	0.1565	0.4315	0.3064	0.2947	0.2724	0.2667	0.9060	0.4315
SCC 6	-0.31467	-0.02456	-0.26922	-0.08548	0.03474	-0.04423	0.34604	0.14735	0.20085	-0.08548
	0.2950	0.9365	0.3737	0.7813	0.9103	0.8859	0.2468	0.6310	0.5106	0.7813
Urea 6	0.00033	-0.53701	-0.51748	-0.36874	-0.02874	-0.35050	0.06685	0.29463	-0.25629	-0.36874
	0.9991	0.0584	0.0701	0.2150	0.9257	0.2403	0.8282	0.3285	0.3980	0.2150
Acetone 6	0.42171	0.45137	0.43062	0.24638	0.39360	0.31591	0.16912	0.39724	-.12114	0.24638
	0.1512	0.1216	0.1419	0.4171	0.1833	0.2930	0.5807	0.1789	0.6934	0.4171
Lactose 6	0.24362	0.06142	0.15706	0.16101	0.04653	0.38529	0.11811	0.22066	-0.04649	0.16101
	0.4225	0.8420	0.6083	0.5992	0.8800	0.1936	0.7008	0.4688	0.8801	0.5992
NFS 6	-0.49589	-0,45693	-0.43160	-0.23143	-0.32085	-0.26888	-0.32896	-0.32792	-0.04635	-0.23143
	0.0848	0.1165	0.1408	0.4468	0.2851	0.3744	0.2724	0.2740	0.8805	0.4468
Fat 12	0.04160	0.54625	0.31798	***0.60634***	0.19913	0.44755	0.06038	***0.65819***	***0.75630***	***0.60634***
	0.8741	0.0233	0.2136	***0.0099***	0.4435	0.0716	0.8179	***0.0041***	***0.0004***	***0.0099***
Protein 12	-0.37388	-0.21098	-0.11838	0.01428	-0.40062	0.01307	-0.44526	-0.02251	0.10674	0.01428
	0.1393	0.4163	0.2136	0.9566	0.1110	0.9603	0.0733	0.9317	0.6835	0.9566
SCC 12	-0.23270	0.31198	0.10678	0.27369	-0.08290	0.20113	-0.8404	***0.48644***	***0.72139***	0.2369
	0.3688	0.2228	0.6834	0.2878	0.7518	0.4389	0.7484	***0.0477***	***0.0011***	0.2878
Urea 12	-0.06537	-0.27186	-0.36781	-0.25715	0.02268	-0.19288	0.02790	-0.08903	-0.20068	-0.25715
	0.8031	0.2912	0.1464	0.3191	0.9312	0.4583	0.9154	0.7340	0.4399	0.3191
Acetone 12	0.14849	-0.15409	-0.22269	-0.32489	0.17125	-0.24042	0.02619	-0.22497	-0.19156	-0.32489
	0.5695	0.5549	0.3903	0.2032	0.5111	0.3526	0.9205	0.3853	0.4614	0.2032
Lactose 12	0.45498	0.50562	0.4700	0.41712	0.33773	0.40559	0.50462	0.29111	0.09747	0.41712
	0.0665	0.0384	0.0569	0.0958	0.1849	0.1063	0.0388	0.2569	0.7098	0.0958
NFS 12	-0.31253	-0.08574	-0.00487	0.15200	-0.35109	0.13704	-0.37654	0.08630	0.19954	0.15200
	0.2220	0.7435	0.9852	0.5603	0.1670	0.5999	0.1363	0.7419	0.4426	0.5603
Fat 24	-0.36885	-0.14536	-0.41991	-0.15674	-0.17018	-0.01499	-0.09211	-0.09226	0.11671	-0.15674
	0.1761	0.6052	0.1192	0.5770	0.5443	0.9577	0.7411	0.7437	0.6787	0.5770
Protein 24	0.15684	0.27508	-0.05820	0.29253	0.28578	0.40552	0.35485	***0.55222***	0.12440	0.29253
	0.5767	0.3211	0.8368	0.2900	0.3018	0.1337	0.1943	***0.0328***	0.6587	0.2900
SCC 24	0.21759	0.39846	0.06227	0.39370	0.32546	0.40527	0.37200	***0.64710***	0.22372	0.39370
	0.4360	0.1413	0.8255	0.1465	0.2365	0.1340	0.1721	***0.0091***	0.4228	0.1465
Urea 24	-0.34897	-0.60627	-0.39927	-0.38778	***-0.69386***	-0.13101	-0.50360	-0.31886	-0.25563	-0.38778
	0.2024	0.0166	0.1404	0.1532	***0.0041***	0.6461	0.0556	0.2467	0.3578	0.1532
Acetone 24	-0.02540	-0.21231	0.17544	-0.22184	-0.30878	-0.33778	-0.40862	-0.46798	-0.06901	-0.22184
	0.9284	0.4475	0.5317	0.4268	0.2628	0.2182	0.1305	0.0785	0.8069	0.4268
Lactose 24	-0.34553	-0.22533	0.05509	0.03035	***-0.61814***	-0.06721	-0.42396	-0.20540	0.11775	0.03035
	0.2071	0.4194	0.8454	0.9145	***0.0140***	0.8119	0.1153	0.4627	0.6760	0.9145
NFS 24	0.08621	0.25313	-0.07129	0.33869	0.18213	0.44938	0.31134	***0.59140***	0.17690	0.33869
	0.7600	0.4194	0.8007	0.2169	0.5159	0.0929	0.2587	***0.0202***	0.5282	0.2169
Fat 36	0.01564	***0.51504***	0.21572	0.48642	0.15759	***0.59326***	-0.12704	***0.76836***	***0.79374***	0.48642
	0.9542	***0.0412***	0.4223	0.0561	0.5600	***0.0154***	0.6392	***0.0005***	***0.0002***	0.0561
Protein 36	-0.16407	-0.30560	-0.28754	-0.24651	-0.06539	0.13801	-0.41033	0.03219	0.19395	-0.24651
	0.5437	0.2497	0.2802	0.3574	0.8099	0.6102	0.1144	0.9058	0.4717	0.3574
SCC 36	-0.31306	-0.38735	-0.34776	-0.29469	-0.26225	-0.12688	-0.37929	-0.07991	0.25221	-0.29469
	0.2377	0.1383	0.1869	0.2679	0.3265	0.6396	0.1474	0.7686	0.3460	0.2679
Urea 36	-0.07476	0.00279	-0.16574	0.0284	0.22175	0.13674	0.37105	0.08852	-0.27561	0.02842
	0.7832	0.9918	0.5396	0.9168	0.4092	0.6136	0.1571	0.7444	0.3015	0.9168
Acetone 36	0.29413	-0.07082	0.23550	-0.19634	-0.08518	-0.43496	0.12072	***-0.58579***	***-0.63215***	-0.19634
	0.2688	0.7944	0.3799	0.4661	0.7538	0.0922	0.6561	***0.0171***	***0.0086***	0.4661
Lactose 36	0.15942	***0.54743***	0.47446	***0.55611***	0.00277	0.27098	0.36567	0.34509	0.16499	***0.55611***
	0.5554	***0.0282***	0.0633	***0.0253***	0.9919	0.3100	0.1637	0.1905	0.5414	***0.0253***
NFS 36	-0.05297	-0.02826	0.00021	0.15340	-0.14729	0.49575	-0.29330	0.37105	0.36318	0.15340
	0.8455	0.9173	0.9994	0.5706	0.5862	0.0508	0.2702	0.1571	0.1668	0.5706

Figure A 6. Broodmare information.

Collar #	Horse	Year	Age	BCS	Rump Fat	%BF
G1	Jane	2005	12	4	0.78	12.31
G4	Bingo	2002	15	5.5	1.34	14.94
G3	Rey	1999	18	6	1.12	13.90
G9	Rita	2012	5	6	0.70	11.93
R7	Deelux	2000	17	4.5	0.72	12.02
R8	Flashy	2001	16	7	1.36	15.03
R14	Facebook	2011	6	4	0.56	11.27
R17	Ditto	2011	6	3.5	0.18	9.49
R16	Bay Recip Mare	2012	5	4.5	0.56	11.27
G11	Vicky	2011	6	4	0.50	10.99
R9	Marla	2003	14	6	0.44	10.71
R20	Streaking Cheval	1999	18	4	0.24	9.77
R19	Reina	2011	6	5	0.38	10.43
G27	Pumpkin	2008	9	6.5	1.55	15.90
B2	Rosey	2002	15	6	0.30	10.71
B1	Twister	2003	14	5	0.44	10.05
B3	Cookie	2007	10	5	0.82	0.82
B4	Missy	2006	11	5	0.86	12.68
B7	Bella	1996	21	4.5	0.64	11.65
B8	Dee	2004	13	6	0.86	12.68
B15	Maurleen	1994	23	6	1.36	15.03