

EFFECT OF LEVEL OF FERMENTABLE NEUTRAL DETERGENT FIBER ON
FEED INTAKE AND PRODUCTION OF LACTATING EWES

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ABSTRACT

The objective of this experiment was to quantify the effect of level of fermentable NDF (FNDF) on DMI and production of highly productive, lactating ewes. Within one week of parturition, 21 ewes and their triplets or twins (2.7 lambs per ewe) were penned individually in expanded metal floor pens and fed one of 3 diets for 6 weeks. The diets were formulated to contain 15, 25, or 35% FNDF with associated decreases in nonstructural carbohydrates based upon estimated ingredient digestibility values at 1X maintenance. The 15% FNDF diet (19% NDF) contained 48.2% corn gluten feed (CGF), 44.9% corn, 2.9% calcium carbonate, 2% mineral-vitamin premix, and 2% corn oil. Soy hulls replaced 3 percentage units (PU) of the CGF and 17 PU of the corn for the 25% FNDF diet (30% NDF), and 6 PU of the CGF and 33 PU of the corn for the 35% FNDF diet (41% NDF). Chromic oxide was used as a marker to determine digestibility. Milk production was measured during week 3 by lamb removal, oxytocin administration, and milking followed 3 hours later by a second milking. Almost all ewes had sore teats by week 4, often followed by mastitis, which was treated with penicillin and udder balm. For ewes fed the 15% FNDF diet, DMI was similar to 2007 NRC values, but DMI was substantially higher for ewes fed the 25 and 35% FNDF diets. Somewhat in line with digestibility depression from increased intake, actual digestibility values were substantially lower than the 1X maintenance values upon which the diets were formulated, but ewe and lamb gains, and milk production increased substantially as dietary FNDF increased. It was discovered that the actual composition of the 15, 25 and 35% FNDF diets was 7, 12 and 16% FNDF but this difference may be attributed to the filter pore size in the method of NDF determination used, causing FNDF to be underestimated. These data indicate that diets for lactating ewes with 2 or 3 lambs should contain a minimum of 12 to 16% FNDF in the dry matter and that diet formulation can have a marked effect on DMI.

BIOGRAPHICAL SKETCH

Melanie Schotthofer was born on May 12, 1983 in Peoria, Illinois. She grew up on a mini-farm, surrounded by horses, sheep, chickens, dogs and cats. When she turned fifteen, her parents changed their jobs and moved to Cochrane, Wisconsin to pursue their dream of a farm. Melanie worked alongside her parents and two sisters to expand a flock of 37 meat sheep into a 450+ ewe flock with dairy sheep genetics and a direct marketing business for the lamb produced. Melanie worked on the farm throughout high school and college, and gained a proficiency and passion for working with sheep. She was especially intrigued with the dairy aspect of sheep. After finishing high school at Cochrane-Fountain City in 2001, she attended the University of Wisconsin-Madison. In 2005, she graduated from UW-Madison with a double major in Animal Sciences and Life Science Communications and Business emphases. A senior research project at Madison brought her into contact with a professor in sheep at Cornell University, Dr. Michael Thonney. Melanie was awarded an assistantship by the Department of Animal Science at Cornell University in 2005 to obtain her masters in Sheep Nutrition. Her interest in dairy sheep led to the study of diets for highly productive lactating ewes.

I dedicate this work first and foremost to my Heavenly Father, who has blessed me greatly, and then to those who believed in me and gave me the confidence to believe in myself – my family, dear friends, and el amor de mi vida, Fernando.

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1. INTRODUCTION

1.1 Introduction

As defined in Basic Animal Nutrition and Feeding, nutrients are chemical elements or compounds in a diet that support normal reproduction, growth, lactation or maintenance of life processes (Pond et al., 1995). Animals require a source of nitrogen in the form of essential amino acids, fat in the form of essential fatty acids, essential mineral elements, a source of energy which may be in the form of fat, protein or fibrous plant tissue as well as some fat and water-soluble vitamins, (Pond et al., 1995).

“The availability of nutrients in a feed is essentially determined by the chemical composition of the feed: first, with respect to the concentrations of available and unavailable components and, secondly, through organic structures and inhibitors that may limit the availability of the components with which they are associated” (Van Soest, 1982).

Nutritionists attempt to quantify the nutrients in a given feedstuff through various feed analysis procedures in order to accurately formulate diets.

1.2 Neutral Detergent Fiber – Measurement and Analysis

Analysis of feed ingredients has developed significantly over the past century. Peter Van Soest, Professor Emeritus, Cornell University contributed immensely to scientific understanding and methods of forage and fiber analysis, which can be accessed in various editions of The Nutritional Ecology of the Ruminant. Van Soest defines true fiber as components of the relatively insoluble cell wall (Van Soest, 1982). The structure of the cell wall is composed of insoluble and soluble substances. The detergent system developed by Van Soest separates forage components into three main classes: the first class contains the cellular contents of soluble carbohydrate, starch, organic acids, protein and pectin, which can all be completely available in the rumen if the rates of passage and digestion allow; the second class represents fractions

with incomplete availability – cellulose and hemicellulose; the third class is completely unavailable – lignins, cutins, silica and other indigestible substances (Van Soest, 1982). Neutral detergent fiber is a measure of the insoluble matrix substances (lignin, cellulose and hemicellulose) because the contents of class I are soluble in the neutral-detergent solution (Van Soest, 1982). Acid detergent is used to digest hemicellulose and fiber-bound protein to measure the amount of cellulose, lignin and lignified N (Van Soest, 1982).

Cellulose, in combination with lignin and hemicellulose, is found in all plant material. Mammals do not have the ability to break down cellulose directly but bacteria within the rumen possess the enzymes required to do so. Hemicellulose, on the other hand, is more easily hydrolyzed by a dilute acid or base and contains many different sugar monomers in contrast to cellulose, which contains only anhydrous glucose (Pond et al., 1995). For instance, besides glucose, sugar monomers in hemicellulose can include xylose, mannose, galactose, rhamnose, and arabinose. Lignin is covalently linked to hemicellulose (Pond et al., 1995). It confers mechanical strength to the cell wall and plays a crucial part in conducting water in plant stems. Lignin is indigestible by mammalian and most other animal enzymes, but some fungi and termites are able to biodegrade the polymer.

1.3 Effects of indigestible NDF and fermentable NDF on growing lambs

Through chemical analyses, the component of a feed that is neutral detergent fiber can be determined. Moreover, if the digestibility of the feed is known, indigestible NDF can be estimated as 100 minus the digestible dry matter minus the metabolic fecal losses (assumed at 10 to 15%) (Hogue, 1999). FNDF can be determined by subtracting INDF from NDF, thus divulging two categories of NDF – a fermentable or digestible component and an indigestible component (Hogue, 1999).

Previous feeding studies at Cornell University indicated that levels or relative proportions of fermentable fiber (FNDF) and nonstructural carbohydrates (NSCHO) may significantly affect dietary intake and performance of highly productive ruminants, (Thonney and Hogue, 2006).

In 1987, Hogue sought to find the quantity of NDF that should be in the diet and how indigestible it should be. His initial studies used two fiber sources- both high in NDF, (soy hulls - 67% NDF and oat hulls- 78% NDF) but the soy hulls were very digestible (estimated INDF 25%) while the oat hulls were very indigestible (estimated INDF 60%) (Hogue, 1987). Diets were fed with varying increments of NDF and INDF to growing lambs; DMI and gain were evaluated. No significant differences in gain were observed, but differences in intake showed that lambs adjusted their intake according to the level of INDF in the diet. The lambs fed the diet with oat hulls exhibited higher intakes than lambs fed soy hull diets with the same level of NDF, but a lower amount of INDF. When comparing diets with nearly equal levels of INDF, almost equal levels of intake were observed. To further test this assumption, different groups of lambs were fed diets with identical amounts of INDF (11%) but from different feed sources (beet pulp, corn gluten feed, wheat midds, alfalfa meal, or oat hulls) (Hogue, 1987). Once again, no significant feed intake differences were observed among the various diets (Hogue, 1987).

Further studies took place to reveal if the relationship between DMI and INDF in the diet held at levels of INDF above 15% of the diet (Hogue, 1991). Again, diets with soy hulls versus oat hulls were used to investigate DMI and gain responses in growing lambs. As diet INDF increased from 15 to 27% in oat hull diets, DMI decreased and INDF intake stabilized. In soy hull diets where the level of INDF increased to a maximum of 20%, (with 51% NDF), feed intake continued to increase linearly, along with proportional increases in NDF and INDF. In further lamb feeding

trials with soy hulls, neither NDF nor INDF appeared to limit feed intake (Hogue, 1991).

In vitro fermentation studies also yielded faster fermentation rates for soy hulls than oat hulls, leading Hogue et al. to conclude that the rate of fermentation or digestion may be a more important factor affecting intake than either total NDF or INDF (Hogue, 1991).

At the Cornell Nutrition Conference in 1994, Hogue reported that ewes 2 to 3 weeks postpartum fed 0.91 kg of hay and allowed *ad libitum* access to high energy complete lamb pellets from Agway, gained weight (130 g average daily gain) during the following 30 days of the trial while nursing triplet lambs. The ewes' average daily intake exceeded the 1985 NRC DMI requirement of 2.73 kg for ewes rearing twins during early lactation by 1.32 kg. This study showed that it is not obligatory for ewes to be in negative energy balance during early lactation, as the NRC requirement tables then indicated.

As reported in a 1999 Cornell Nutrition Conference, Thonney and Hogue observed gain and DMI of 50 day old lambs fed 14, 19 and 24% INDF diets where soy hulls versus oat hulls were used in combination with corn, soybean meal and minerals (1999). In this experiment, DMI was unrelated to the NDF concentration in the diet and consequently, the soy hull diets with 24% INDF had much higher NDF concentrations than the 24% INDF oat hull diets. As expected, as INDF concentration increased in oat hull diets from 14 to 19%, DMI decreased (3.6 to 3.5% of body weight) whereas DMI continued to increase (4.0 to 4.2% of body weight) with increased INDF (14 to 19%) from soy hulls. See Figure 1. Unexpectedly, a diet with 24% oat hulls and 27% soy hulls (24% INDF) elicited the highest feed intake (4.6% of body weight). The authors concluded that the rapid fermentation of soy hull diets may

compensate for the expected lower digestibility of the additional oat hulls to allow for the increased feed intake observed, (Thonney and Hogue, 1999).

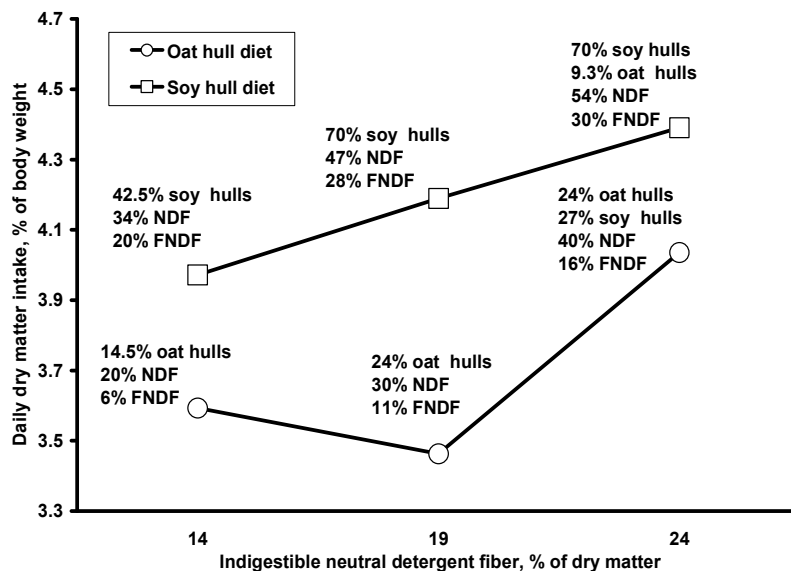


Figure 1.1 Relationship of daily dry matter intake of growing lambs to dietary INDF concentration, revised from (Thonney and Hogue, 1999).

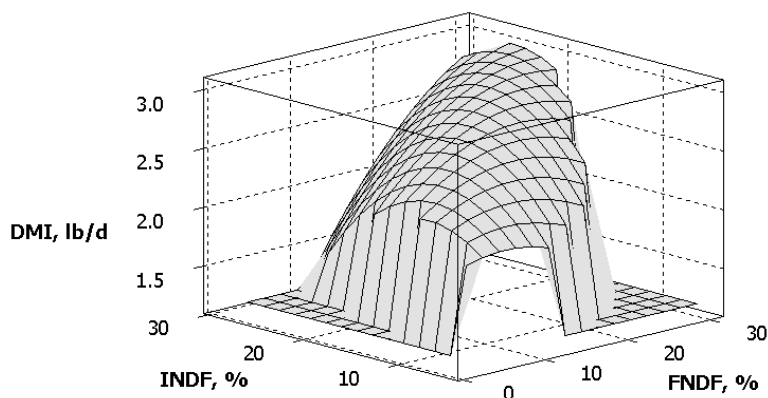


Figure 1.2 Surface plot showing the equation that describes the relationship of feed intake to dietary INDF and FNDF concentrations. The equation was $DMI = 1.59 + 0.1014 \cdot INDF + 0.00610 \cdot INDF \cdot FNDF - 0.00228 \cdot FNDF^2 - 0.00584 \cdot INDF^2$ with $SE = 0.14$ and $r^2 = 0.85$ (Thonney and Hogue, 2006).

The aforementioned studies demonstrate that the relationship of feed intake to dietary fiber depends upon the fermentability of the fiber. In Figure 2, INDF and

FNDF explain 85% of the variation in a diet mean feed intake of the experiments discussed previously (Thonney and Hogue, 2006).

1.4 Effects of soy hulls on the digestibility of NDF

Soybean hulls have been used as a source of NDF for each of the lamb feeding experiments described, including the one represented by this thesis. Therefore, it is important to note the impact soy hulls have on NDF digestion and rumen fermentation. Although soy hulls contain high levels of NDF usually associated with forages, soybean hulls have differing physical and chemical composition that translates into dissimilar effects in the rumen. For instance, soy hull fermentation does lead to high levels of acetate in the rumen, but due to a low effective fiber content (2% of NDF according to the 1996 NRC), rumination is not greatly stimulated by soybean hulls (Titgemeyer, 2000). Moreover, chemical composition of soybean hulls can be subject to wide amounts of variation due to the degree of cleaning the by-product feed undergoes; well-cleaned soy hulls contain 9.4% crude protein and 64% NDF (Titgemeyer, 2000). When soybean meal is not completely cleaned out, the percentage of protein in the soy hulls can increase and NDF levels decrease (Titgemeyer, 2000). Soy hull NDF is largely composed of cellulose and due to its low (1.8 to 3.9%) lignin levels, soy hull NDF undergoes rapid and extensive fermentation or digestion (Titgemeyer, 2000). Also, studies cited by Titgemeyer (2000) note that when soybean hulls are fed as the sole or major ingredient in the diet, such as in the Thonney and Hogue diets, *in vivo* digestion of soybean hulls is found to be considerably less than that of *in vitro* digestion rates, suggesting that the rate of passage is too high to allow sufficient time for digestion in the rumen. “The rapid passage rate can be attributed to the small particle size of soybean hulls and the relatively high specific gravity” (Titgemeyer, 2000). Thus, a method to improve the

extent of ruminal digestion of soy hull NDF would be to decrease the ruminal passage rate. This can be done through the addition of long or effective fiber, which requires more time to be digested into adequately sized particles to move out of the rumen. Another impact on passage rate is DMI; as intake increases, passage rate increases and certain processing like fine grinding of soybean hulls only exacerbates the depression in ruminal digestion due to increased passage rate (Titgemeyer, 2000).

In evaluating the performance of finishing steers, soybean hulls added to grain-based diets by Ludden et al., 1995 brought about similar responses in gain and DMI as those seen by Hogue and Thonney (1999) and Titgemeyer (2000). When soy hulls replaced corn for up to 60% of the diet, linear decreases in average daily gain ($P = 0.03$) and gain efficiency ($P < 0.001$) were observed as DMI increased ($P = 0.03$) with the addition of soy hulls. The authors attributed the decrease in gain to the decreased feeding value of soy hulls in comparison with corn (Ludden et al., 1995). When Ludden et al. compared *in situ* digestion of NDF from different sources in cows fed corn-based diets, they found rapid disappearance of NDF from soy hulls (6.1%/h) and negligible NDF disappearance for alfalfa (0.5%/h) (1995). The authors believed this might indicate that an acidic ruminal environment, such as what may be caused by a corn-based diet, may have more prominent negative effects on the digestion of supplemented forages than on supplemented high-fiber by-products like soy hulls (Ludden et al., 1995).

Soybean hulls do not appear to greatly inhibit digestion and intake of forages, likely due to the microbial population they stimulate, which is the same fiber-fermenting (structural carbohydrate or SC) bacteria that digest forage (Titgemeyer, 2000). However, when added as a supplement to forage in a diet, soybean hulls decrease forage intake while increasing total DMI and diet digestibility (Titgemeyer, 2000) likely due to the more rapid digestive ability of soy hulls comparatively.

Grain-based concentrates stimulate the nonstructural carbohydrate (NSC) bacteria; thus, when fed with soy hulls, the different (SC and NSC) microbial populations compete for nutrients, which can decrease SC digestion (Titgemeyer, 2000). Moreover, NSC bacteria grow at much faster rates than structural carbohydrate (SC) bacteria, enhancing the competition.

1.5 Model accounting for digestion rates in ration formulation

The Cornell Net Carbohydrate and Protein System (CNCPS) is a model developed to predict requirements, feed utilization, animal performance and nutrient excretion for dairy cattle, beef cattle or sheep, using accumulated knowledge about feed composition, digestion, and metabolism in supplying nutrients to meet requirements (Chase et al., 2007). As opposed to the NRC and other formulation systems and models, CNCPS does not balance according to the amounts of specific feed components, but rather predicts their degradation in the rumen (Russell et al., 1992). CNCPS has a submodel that predicts different rates of feedstuff degradation as well as rates of ruminal passage as a function of DMI, particle size, bulk density, level of intake and type of feed (Sniffen et al., 1992). The extent of ruminal digestion and energy availability of a given feed substance is reduced at increased rates of passage (Sniffen et al., 1992). Since soy hulls exhibit a high rate of degradation in *in situ* and *in vitro* experiments, this type of model proves useful in accounting for the effects of using soy hulls as a source of NDF versus other NDF sources. A set of companion papers (Russell et al., 1992) and (Sniffen et al., 1992) demonstrate that the CNCPS equations can be used to accurately predict rumen bacterial growth under certain conditions, two of which include: 1) carbohydrates are divided into structural (SC) and nonstructural (NSC) components, and 2) degradation rates of SC and NSC components can be estimated (Sniffen et al., 1992). In interpretation, the CNCPS

model can use levels of FNDF or proportions of FNDF in the diet relative to other ingredients and INDF, to predict rumen bacterial growth, which determines the relative concentrations of volatile fatty acids (VFA's) as well as the rumen environment. The amount of VFA's produced in the rumen indicates the animal's energy status and production capabilities, and the ratios and proportions of VFA's affect milk composition. However, CNCPS does not use levels of digestible NDF, or FNDF, to predict dry matter intake, as was the goal of this experiment.

1.6 Basis for diet formulation of traditional systems

Current feed formulation guidelines such as the NRC and the ARC are based upon first deriving the nutrient requirements for a given animal in a given stage of production, and then formulating a balanced diet with ingredients of known nutrient composition to fulfill the animal's predicted nutrient requirements. Current systems of forage analysis and ration formulation such as the NRC specify minimum fiber levels as a given percentage of forage or NDF without regard to the digestibility of the fiber, (Thonney and Hogue, 2006). Dado and Allen observed increased (1.9 kg/d) milk production ($P < 0.02$) and increased (1.0 kg/d) DMI ($P < 0.01$) in cows fed an alfalfa silage diet with a higher NDF digestibility compared to an alfalfa silage diet with the same NDF concentration but lower NDF digestibility (Dado and Allen, 1996). Cannas found that dairy ewes in mid lactation increased milk yields and DMI more when fed diets with high digestible fiber than high starch diets but he found the reverse effects in early lactation: the high starch diets elicited higher milk yields and increased DMI (Cannas, 2006). Thus, disregarding the digestibility of fiber or the amount of digestible fiber when formulating a diet can have strong impacts on production.

Traditional feed formulations also generate a value for recommended DMI for an animal at a given stage of production, ignoring the role individual feed components

or their interaction may have on DMI. In a study that investigated constraints on ruminant voluntary feed intake of forages, Allen et al. agreed with Waldo's suggestion in 1986 that NDF is the best single chemical predictor of voluntary DMI (Allen, 1996). However, without accounting for other factors such as initial particle size, particle fragility, chewing frequency and effectiveness, rate of fermentation of the potentially digestible NDF, indigestible NDF and characteristics of reticular contractions, NDF alone cannot adequately predict voluntary DMI (Allen, 1996).

1.7 Premises of the Dugway Formulation System

The previous feeding studies at Cornell University have elucidated problems with traditional feed formulations and given evidence to support a different type of formulation method for highly productive ruminants that redefines dry matter intake and maintenance requirements by balancing for minimum levels of FNDF and maximum levels of nonstructural carbohydrates (NSCHO) in the diet. The method of formulation tested in this experiment which strives for this balance is called the Dugway System, developed by Doug Hogue, Professor Emeritus, Cornell University and Michael Thonney, Professor, Cornell University. It balances the fermentable portion of neutral detergent fiber (NDF) against NSCHO, working under the premise that there should be a minimum level of FNDF and a maximum level of NSCHO (Thonney and Hogue, 2006). Moreover, formulation is based upon the feed components that make up the energy portion of a diet rather than a pooled energy value due to the premise that ingredient composition of a diet influences DMI so that balancing diets based upon assumed intake levels is difficult.

1.8 Basis of the Dugway Formulation System

The Dugway System does not estimate Total Digestible Nutrients (TDN), Metabolizable Energy (ME), Net Energy (NE) feed values or animal requirements, but uses indigestible (INDF), fermentable (FNDF), and NSCHO fractions. This allows the effects of individual components of the diet to be considered for maintaining rumen health and controlling feed intake. Secondly, diet formulation is based on the level of production, and the composition of the diet is assumed to be a primary determinant of dry matter intake (DMI), (Hogue, 1999).

Ingredients and component levels considered are FNDF, INDF, NSCHO, CP (crude protein), Fat and Ash. The sum of these components is 100% of the diet.

1.9 Experimental objectives

Although Hogue and Thonney developed preliminary suggested component levels for FNDF and INDF in the diets (Figure 1.3), further research was needed to better define the minimum FNDF levels and corresponding NSCHO levels in diets for highly productive lactating ewes.

The purpose of this experiment was to study the effects of different ratios of FNDF to NSCHO on production criteria in ewes with triplets in hopes of recommending a minimum FNDF level. Moreover, to account for decreases in digestibility of fiber and fiber components in feeds at levels of intake above maintenance, Van Soest published “Discounts for net energy and protein – fifth revision”, (Van Soest et al., 1992). Since the Dugway System plans to incorporate these values in the future, the discount values were used when evaluating results to compare with actual digestibilities measured.

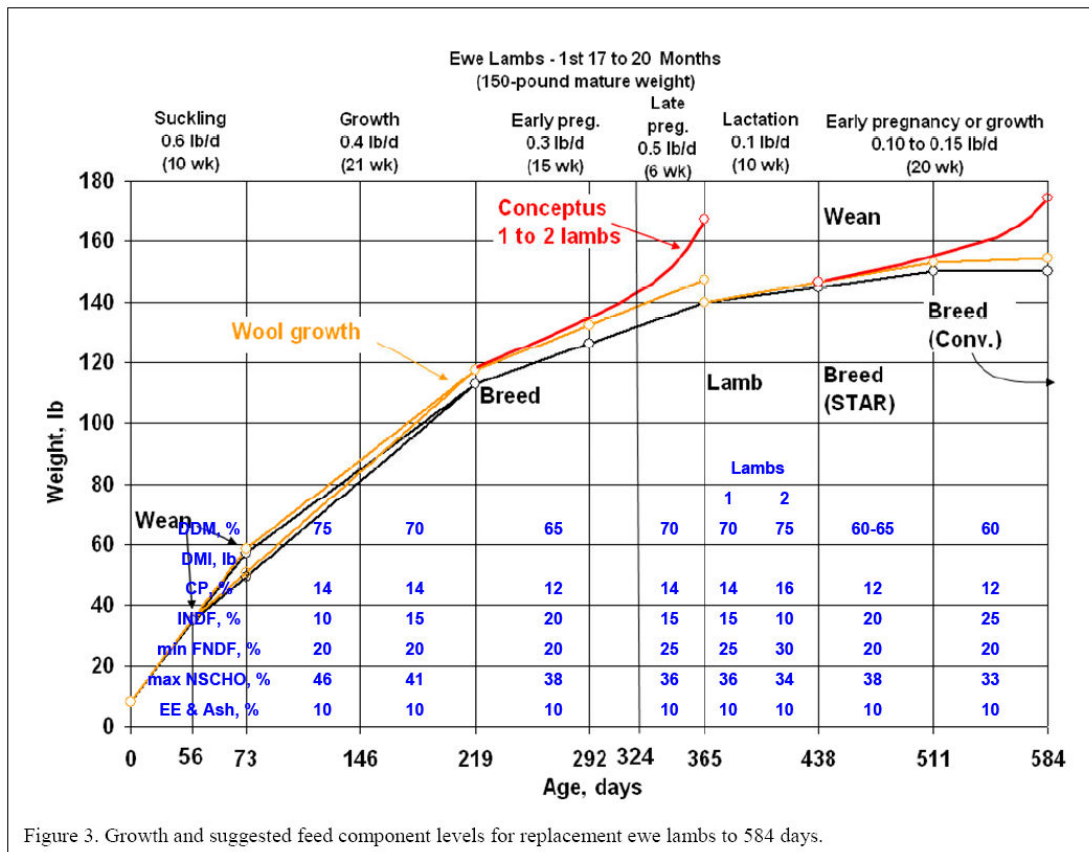


Figure 3. Growth and suggested feed component levels for replacement ewe lambs to 584 days.

Figure 1.3 Growth and suggested feed component levels for replacement ewe lambs to 584 days.

STATEMENT OF PURPOSE

The following feed trial was designed to quantify the ratio of nonstructural carbohydrates to fermentable neutral detergent fiber in a diet that results in the most productive lactating ewes in terms of body weight maintained, pounds of lamb produced, pounds of milk produced and feed efficiency. The study was also conducted to quantify the various effects on blood metabolites, milk composition and rumen dynamics of feeding a diet where the sole ingredients and sources of fiber are soybean hulls, corn gluten feed and corn.

2. MATERIALS AND METHODS

2.1 EXPERIMENTAL ORGANIZATION

Twenty-one Finnsheep x Dorset ewes with twins or triplets from the Cornell University Teaching and Research Center flock were randomly assigned within a week of lambing to diets that contained 15, 25, or 35% FNDF, assuming ingredient digestibility values at 1 X maintenance. During the experiment, all ewes were allowed to consume feed *ad libitum*. They also received water *ad libitum*. No additional feedstuffs were fed to the ewes throughout the 6 week trial.

There was no preliminary adjustment period to the diets as the ewes all had *ad libitum* access prior to the experiment to a 28% FNDF diet that was very similar in composition to the 25% FNDF diet (Table 2.1.1).

Ewe weights, lamb weights, blood samples and feed weighbacks for ewe feeds and creep feeds were collected every Tuesday. The third week of the experiment, which was approximately the fourth week of lactation for the ewes, milk measurements were taken for yield and components. Starting the fourth week of the experiment, lambs were offered creep feed, which was identical to the mother's diet. On April 18, 2006, which corresponded with the fifth week for pens 10, 14 through 17 and 20, the fourth week for pens 1, 2, 3, 5, 6, 7, 8, 9, 11, 12, 13, 18, 19 and 21, and the third week for pen 4, lambs were also given hay to promote creep feeding versus nursing due to developing udder sores on the ewes. Chromic oxide was mixed with each diet and fed to the ewes for 7 days, beginning the fifth week of the trial to determine digestibility. Feces were sampled on day 7 after the commencement of feeding the marker.

Ewes and their lambs returned to the flock following the completion of the experiment.

2.1.2 Diet preparation

Table 2.1.1 Diet composition¹

Ingredients, % DM	Pre-experiment diet ²	15% FNDF	25% FNDF	35% FNDF
Corn grain	25.1	44.9	27.9	11.7
Corn gluten feed	41.6	48.2	45.2	42.3
Soy hulls	27.2	---	20.5	40.1
Vegetable oil	---	2.0	2.0	2.0
Premix – Mercer Milling ³	2.20	2.0	2.0	2.0
Calcium carbonate	2.27	2.9	2.4	1.9
Molasses	1.68	---	---	---
Expected nutrient composition				
DM, %	89.5	90.0	90.0	90.1
DDM, %	80.7	79.3	78.8	78.4
CP, %	15.8	16.0	16.0	16.0
NDF, %	33.7	19.4	30.5	41.1
Indigestible NDF, %	5.73	4.84	5.47	6.08
Fermentable NDF, %	28.0	14.6	25.0	35.0
NSCHO, %	39.6	50.7	40.1	30.1
Ether extract, %	4.46	7.38	6.90	6.45
Ash, %	6.43	6.55	6.49	6.40
Calcium, %	1.14	1.36	1.24	1.12
Phosphorus, %	0.57	0.67	0.61	0.55
Potassium, %	1.23	0.98	1.14	1.30
Magnesium, %	0.29	0.27	0.28	0.30
Sulfur, %	0.05	0.18	0.17	0.16
Iodine, ppm	0.90	0.87	0.86	0.85
Iron, ppm	186	84.4	159	231
Copper, ppm	5.76	3.11	4.10	5.05
Molybdenum, ppm	0.72	0.71	0.74	0.76
Cobalt, ppm	0.40	0.38	0.37	0.37
Manganese, ppm	53.7	54.5	53.8	52.9
Zinc, ppm	60.9	56.9	60.4	63.8
Selenium, ppm	0.34	0.34	0.34	0.34
Vitamin A, kIU/lb	1.29	1.28	1.28	1.28
Vitamin D, kIU/lb	0.17	0.17	0.17	0.17
Vitamin E, IU/lb	10.4	10.3	10.3	10.2
Decoquinatate, g/lb	0.015	0.015	0.015	0.015

¹Formulated based on the Dugway system using FeedForm.

²Ewes were all fed this diet *ad libitum* along with some hay for approximately 2.5 months before lambing.

³Composition shown in Table 2.1.2.

The diets were formulated based upon feed analysis values and calculation of FNDF for individual ingredients to obtain desired compositions shown in Table 2.1.1. Diets were mixed at the Cornell Sheep Farm and stored in separate, labeled bins. During the week of feeding chromic oxide, chromic oxide was mixed with the diets at approximately 0.5% of the diet. The diets with chromic oxide were stored separately.

Table 2.1.2 Mercer Milling premix composition, January 2006.

Item	Units	Premix	Diet
Ammonium chloride	%	37.5	0.750
Salt	%	24.9	0.499
Deccox, ¹ 6% concentrate	%	2.42	0.048
Calcium	%	0.129	0.003
Potassium	%	0.013	0.0003
Sulfur	%	0.130	0.003
Iodine	ppm	40.0	0.800
Cobalt	ppm	7.33	0.147
Iron	ppm	12.6	0.251
Manganese	ppm	1,504	30.1
Selemium	ppm	15.0	0.300
Vitamin A	kIU/kg	25.7	0.514
Vitamin D	kIU/kg	3.45	0.069
Vitamin E	kIU/kg	206	4.11

¹Decoxx is a common coccidiostat that interrupts the life cycle of coccidia larvae, thus improving feed efficiency.

2.1.3 Diet feeding

The first day of the experiment, 4.5 kg of feed was placed into each ewe's individual feed bin. The following days, the amount of fresh feed added depended on the ewe's intake from the day before, but efforts were made to keep feed available at all times so ewes could eat *ad libitum*. Each week, feed weighbacks were taken and a fresh 4.5 kg of feed was given to each ewe.

2.1.4 Ewe selection

Prior to ewe assignment, the diets were assigned randomly to pens 1 through 21. Ewes with triplets were selected based on the viability of their triplets, the

qualifying criteria being that the triplets needed to appear strong enough within a week of birth to survive without supplementation. Ewe assignments occurred over a 3-week time period. The first week, March 21, 2006, 7 ewes were ready for the experiment based on the qualifying conditions. They were randomly placed in pens under infrared lamps in pens 10, 13, 14, 15, 16, 17 and 20. The pens consisted of raised 1.2 x 2.4 meter pens. One ewe with her 3 lambs was assigned to one raised pen. Ewes and lambs were weighed and an initial blood sample was taken for each ewe. The second week, March 28, this was repeated for a second group of ewes and their lambs; they were assigned to the remaining pens: 1 through 9, 11, 12, 18, 19 and 21. The ewe in pen 13, P2237 (15% FNDF) had to be removed by the second week because her feed intake dropped to a negligible amount and she lost considerable weight; her lambs appeared hungry as well. She was replaced with CXB3432. By March 30, F3606 in pen 4 (15% FNDF) was removed due to feed refusal and the death of one of her triplets. Thus, the third week, April 4, CXB3690 replaced F3606 in pen 4 and began the experiment. Due to a shortage of ewes with triplets that met the viability criteria within the necessary time frame, 6 ewes with twins were used, resulting in 5 ewes with triplets and 2 ewes with twins assigned to each of the 3 diets.

2.2 MEASUREMENTS TAKEN

2.2.1 Body weight

Ewes and their lambs were weighed before beginning the trial and every week thereafter for a total of 7 weights.

2.2.2 Feed intake and feed samples

Feed intake was measured weekly by subtracting feed remaining from the amount fed to each ewe during the week. Feed samples were collected 4 times

throughout the experiment (March 31, April 4, April 14, and April 18) and chromic oxide-treated feed samples were collected April 21 and April 25. The samples were ground through a 1 mm screen in a Wiley mill and then stored in a capped glass jar.

Dry matter values were determined on all samples by hotweighing after leaving the samples in a 106°C oven overnight. The hotweighing procedure consisted of first placing empty crucibles in a 106°C oven for 2 to 3 hours. Then, individual empty crucibles were taken directly from the oven and placed in a scale with sliding doors. Hot samples first lose weight and then gain weight in less than a minute. The weight was recorded at the lowest possible weight. The crucible was removed and the next crucible was weighed after taring the scale. Later, an exact amount of feed or fecal sample was weighed onto a piece of weighing paper in a 4 place scale. The sample was then poured into the previously weighed crucible and the weight of the weighing paper and any residue still remaining on it from the sample was subtracted from the initial weight. The crucible with sample was placed in the 106°C oven for 3 hours, after which the hotweighing procedure was repeated. The percentage of dry matter content was determined by:
$$\frac{[(\text{hotweight of crucible with sample} - \text{empty crucible hotweight}) / (\text{sample weight})] \times 100$$

2.2.3 Fecal samples

Ewes were fed the feed with chromic oxide during the fifth week of the experiment. Six days later, a plastic tarp was spread underneath each pen. Forty-eight hours later, feces were collected from throughout the tarp to fill 2 loaf pans and the feces in the loaf pans were dried at 140°C. Weights were recorded daily and a fecal sample was considered dry when it stopped losing weight. Fecal samples were later ground through a 1 mm screen in a Wiley mill and stored in glass jars. Prior to laboratory analysis, dry matter values were determined on all samples by the hotweighing procedure described in 3.2.2 after drying them in a 106°C oven overnight.

2.2.4 Blood samples

Blood was collected from each ewe at the start of the experiment and at the end of each of the following 6 weeks. Approximately 10 mL of blood was drawn via jugular puncture and subsequently centrifuged for 20 min at 4°C and 2500 x g in a Beckman Coulter Allegra 6R centrifuge; plasma was divided into 5 aliquotes. Two aliquotes contained 0.05 mL of 5.8 M perchloric acid to deproteinate 0.45 mL of plasma designated for lactic acid assays. Plasma was frozen at -20°C until assayed for plasma urea nitrogen, beta-hydroxybutyrate, non-esterified fatty acids, L-lactic acid and D-lactic acid.

2.2.5 Milk samples

Ewes were separated from their lambs by placing a divider into the pen. Each ewe received an intramuscular injection of 0.25 mL of oxytocin (20 USP Posterior Pituitary units per mL) in the neck. Immediately after the injection, the ewe was milked out completely and the milk was discarded. Exactly 3 hours after milking, the injection of 0.25 mL of oxytocin was repeated and the ewe was completely milked out again. The milk was weighed and 2 samples were taken. One sample from each ewe was taken to Dairy One Laboratory for composition analysis. Milk weights were multiplied by 8 to convert milk yield to a 24-hour basis.

2.3 ANALYSES

2.3.1 Blood metabolite analyses

Beta-hydroxybutyrate

An adaptation of a Sigma Kit procedure #310-UV (St. Louis, MO) was used to perform the assay. See Appendix I for the complete procedure.

D-Lactic acid and L-Lactic acid

After thawing, the plasma was centrifuged for 10 min at 4°C and 2500 x g in a Beckman Coulter Allegra 6R centrifuge. Supernatant (0.3 µL) was transferred to a microfuge tube for each sample. Next, the supernatant was neutralized with 6 M KOH (0.0525 µL) and vortexed. After 10 min of refrigeration, samples were centrifuged for 10 min using the same microcentrifuge at 4°C and 2500 x g. Next 300 µL of the supernatant was transferred into a 1.5 mL tube. The sample was frozen until assayed.

The protocols for the L and D-lactic acid assays were adapted from (Bryk-Lucy, 2000) which was adapted from Sigma Kit 826-UV (St. Louis, MO) for measuring L-lactic acid. 96-well plates were used in place of microcuvettes. See Appendix I for the complete procedure.

Non-esterified fatty acids (NEFA)

Enzymatic analysis using NEFA-C Reagents from WAKO (Richmond, VA) was used to perform the assay with the following adaptation. Reagents A and B were further diluted and used in proportion to each other. See Appendix I for the complete procedure.

Plasma urea nitrogen (PUN)

PUN's were performed in the laboratory using a method based on Sigma Kit No. 640 (St. Louis, MO). See Appendix I for the complete procedure.

2.3.2 Milk analyses

Milk was analyzed by Dairy One Laboratory for percent milk fat, true protein, crude protein, lactose, somatic cell counts, milk urea nitrogen, and total solids.

2.3.3 Feed, fecal and digestibility analyses

Chromium determination – Chromium was measured with methods from Ruiz (2001), which was a modification of method 984.27 of AOAC (1990).

Approximately 0.2 g of a previously dried and ground feed or fecal sample was ashed in a muffle furnace at 520°C for 2 h in a 125 mL Ehrlenmeyer flask. After cooling, 4 mL of double-distilled nitric acid was added in a perchloric acid hood and digested for 1 h on a hot plate set at 120°C. Then, 10 mL of 70% perchloric acid was added to each flask and the temperature was gradually raised to 220°C. Samples were refluxed for approximately 0.5 h until oxidized. The oxidation point was determined when the green (Cr III) turned to orange (Cr VI). Cooled samples were transferred quantitatively to 100 mL volumetric flasks and filled to the 100 mL volume with double-distilled water. The final acid content of the solution was about 10 percent. The samples were then taken to the Cornell Nutrient Analysis Laboratory (804 Bradfield Hall) where they were diluted 1:1 and analyzed by inductively-coupled plasma emission spectroscopy for chromium concentration.

NDF Analyses

NDF analyses were performed according to Mertens (2003) and Van Soest et al. (1991). Briefly, a 0.5 g sample was weighed and placed in a 600 mL beaker with 100 mL neutral detergent, 200 µL heat-stable amylase (Termamyl[®]; Novo Nordisk Bioindustrials, Inc., Danbury, CT) and approximately 2.46 cm³ sodium sulfite. Samples were refluxed on the burners exactly 1 h after boiling commenced. Then, each beaker was filtered through a pre-hotweighed crucible containing a glass microfiber filter (Whatman 934-AH; Whatman Inc. Clifton, NJ) under vacuum. Each crucible was rinsed 3 times with boiling water. If a sample required an exceptionally long filtering time, as was true for many of the samples with chromium, an additional 200 µL of amylase was added. After the third rinse, the crucible was lightly coated with acetone and then allowed to dry. Samples were then hotweighed, ashed and hotweighed again. NDF was the difference between the hotweights.

ADF/Lignin

First, Acid Detergent Fiber was determined according to (Van Soest et al., 1991). One g of sample and 100 mL of Acid Detergent (AD) solution was added to a 600 mL beaker and refluxed for 1 h. It was filtered under vacuum, rinsed 3 times with boiling water, rinsed with acetone, dried and hotweighed. Sulfuric acid (72%) was added to the AD residue at room temperature, where it was soaked for 3 h, stirring at every hour. After 3 h, the crucible was rinsed with boiling water 5 times under vacuum. It was then placed in a 106°C oven overnight and ashed at 520°C for 3 hours. The lignin was hotweighed. Calculation of lignin was:

$$\{[(\text{Lignin hotwt} - \text{Empty hotwt}) - (\text{Ash hotwt} - \text{Empty hotwt})]/(\text{Sample wt, \% DM})\} \\ \times 100$$

Fermentation Rates

A 0.5 g sample was measured into a 125 mL Ehrlenmeyer flask containing 40 mL of fermentation buffer. Ten mL of rumen fluid from a fistulated dry cow was added to the flask and incubated at 39°C for 6 h and 24 h, 2 flasks per sample. Once the designated fermentation time was complete, the respective samples were removed and NDF was assayed (Goering and Van Soest, 1970).

2.3.4 Statistical analyses

Blood metabolite data were analyzed by univariate repeated measures ANOVA in JMP 6.0.3 (Cary, NC) with the following effects: diet, ewe within diet as a random effect, week, diet by week interaction and random error. Ewe within diet was the error term for diet and random error was the error term for week and diet by week interaction. Health data were analyzed with Chi Square Analysis by Minitab (State College, PA). All other data were analyzed by one-way analysis of variance using the General Linear Model procedure of Minitab (State College, PA). Orthogonal contrasts were used to find significance between diets. Orthogonal contrasts were 15% FNDF

versus the average of the 25% FNDF and 35% FNDF diet means and 25% FNDF versus 35% FNDF. Effects were considered to be significant at $P < 0.05$ unless otherwise stated.

2.4 CALCULATIONS

2.4.1 Calculating digestibility

Diet digestibility was determined using the relationship between the concentration of chromic oxide in the feces and the concentration of chromic oxide in the feed. Ewe Pu286 in pen 1, fed 25% FNDF feed will be used as an example to illustrate the derivation of the digestibility and digestible NDF values. Ewes in pens 1 to 9, 11, 12, 18, 19 and 21 were fed the diets sampled on April 25, while ewes in pens 10, 13 to 17 and 20 were fed the diets represented by samples taken on April 21.

The Cornell Nutrient Analysis Laboratory provided the values for concentration of chromic oxide in the feces and feed. Each sample was assayed in duplicate; for the ewe in pen 1, Pu286, the values were:

25% 4-25-06 feed: 9.903 mg Cr/L

Pen 1 feces: 22.159 mg Cr/L

Duplicate: 8.672 mg Cr/L

Duplicate: 21.742 mg Cr/L

The average of the duplicate values was used in the example to calculate feed digestibility for the ewe in pen 1, Pu286, which was fed the diet with 25% fermentable neutral detergent fiber.

Therefore, the 25% FNDF feed mixed with chromic oxide and fed to Pu286 in pen 1 was 54.7% digestible in that ewe. Equation (9) above is derived as follows:

$$\text{Indigestibility} = (\text{g feces/mg Cr}) \times (\text{mg Cr/g feed}) = (\text{g feces/g feed})$$

$$\text{Digestibility} = 1 - \text{indigestibility}$$

Table 2.4.1 Calculation of feed digestibility using feed sample (25% FNDF 4-25-06) and fecal sample from Ewe Pu286 in pen 1.

Item	Feed ^a	Feces ^b
(1) Sample [Cr] of solution, mg/L	9.29	21.95
(2) Liters of solution after Cr hydrolyzation	0.20	0.20
(3) Cr in sample, mg [(1) x (2)]	1.86	4.39
(4) Sample weight, g	0.205	0.205
(5) Percent dry matter/100	0.90	0.96
(6) Sample DM, g [(4) x (5)]	0.185	0.197
(7) mg Cr per g feed or fecal DM [(3)/(6)]	10.05	22.28
(8) Cr as a percent of feed or fecal DM [(7) x 0.1]	1.01	2.23
(9) Digestible DM, % $\{1 - [(8^a)/(8^b)]\} \times 100$		54.7

2.4.2 Calculating NDF

NDF digestibility was calculated as follows.

$$\text{mg Cr/g feed NDF} = (\text{g feed DM/g feed NDF}) \times (\text{mg Cr/g feed DM})$$

$$\text{mg Cr/g feces NDF} = (\text{g feces DM/g feces NDF}) \times (\text{mg Cr/g feces DM})$$

$$\text{NDF indigestibility} = (\text{g feces NDF/mg Cr}) \times (\text{mg Cr/g feed NDF})$$

$$\text{Digestibility} = 1 - \text{NDF indigestibility}$$

Values of digestible NDF are consistent with those calculated using Van Soest's equation on page 42 of The Nutritional Ecology of the Ruminant.

Table 2.4.2 Calculation of NDF in feed or fecal samples using feed sample 25% FNDF, taken 04-04-06.

Item	25% FNDF
(1) Sample weight, g	0.501
(2) Percent dry matter/100	.91
(3) Dry matter sample wt, g (1) x (2)	0.456
(4) Empty crucible weight, g	36.36
(5) Filtered NDF hot weights, g	36.52
(6) Ashed weight, g	36.36
(7) Grams of NDF sample/g of sample DM [(5)-(4)]/(3)	0.351
(8) Ash sample g/g of sample DM [(6)-(4)]/(3)	0.00
(9) Ash corrected NDF, g/g of sample DM [(7)-(8)] x 100	35.1%

3. RESULTS AND DISCUSSION

Table 3.1 Diet analysis by Dairy One Laboratory

Analyzed nutrient composition	15% FNDF	25% FNDF	35% FNDF
DM, %	91.8	90.6	90.8
NE _L ¹ , Mcal/lb	0.87	0.83	0.80
CP, %	17.0	17.4	17.3
NDF, %	16.6	28.6	38.0
NDF ² , %	23.2	32.7	42.4
Calcium, %	1.52	1.49	1.29
Phosphorus, %	0.52	0.50	0.45
Magnesium, %	0.25	0.28	0.29
Potassium, %	0.85	1.05	1.17
Sodium, %	0.30	0.34	0.33
Iron, ppm	138	202	262
Zinc, ppm	55	61	64
Copper, ppm	8	11	13
Manganese, ppm	54	50	50
Molybdenum, ppm	<0.1	<0.1	0.2

¹NE_L, Mcal/lb = (((81.41 – (0.6 x (ADF% x 0.80))) x 0.0245) – 0.12) x 0.454

²This value was measured in the Cornell Department of Animal Science NDF lab. The discrepancy between this value and the NDF value from Dairy One is due to a difference in filters used. The F57 filter bags used in the Ankom method used by Dairy One had a porosity of 25 microns whereas the glass microfiber filters used in the Cornell NDF lab had a porosity of 1.5 microns.

Dairy One Laboratory analyzed the diets to provide composition data in Table 3.1. Crude protein was fairly constant among all diets in order to eliminate the effects of varying protein levels. The NE_L values of the diets shown in Table 3.1 exhibit a decrease with the corresponding increase in level of FNDF, indicating that the diets were more energy concentrated in the lower FNDF diets. These diets had a higher concentration of corn grain, which explains the higher energy values seen while the 35% FNDF diet contained less corn and more soy hulls.

The discrepancy between the NDF value arrived at by Dairy One using the Ankom method and the NDF value derived in the Cornell Department of Animal

Science lab using the standard NDF procedure by Mertens and Van Soest can be attributed to the difference in porosity of filters used. At the Cornell lab, samples were refluxed in 600 mL beakers and then filtered through crucibles with glass microfiber filters (Whatman 934-AH; Whatman Inc. Clifton, NJ) with porosity of 1.5 microns, whereas at the Dairy One lab, samples were placed in a F57 filter bag with porosity of 25 microns and the bag with sample was refluxed in neutral detergent solution. While the larger micron size may potentially allow the escape of more particles, leaving less residue to be characterized as NDF and explaining the lower NDF values from the Dairy One lab, it is more likely that the 1.5 micron porosity of the filters used in the Cornell NDF lab were too small to allow all the cell solubles to filter out, and NDF was overestimated. The incredibly fine particle size of the chromium in feed and fecal samples used to determine digestibility likely compounded this effect by blocking the filter pores. It was noted in Materials and Methods that the samples with chromium took extra time and amylase to filter through, thus supporting this theory. Therefore, the NDF values from the Cornell lab, which were used to evaluate the diets in this thesis, were likely overestimated.

Table 3.2 In vitro fermentation rates of diet ingredients

Ingredients	Hours fermented	NDF, % DM	Lignin, % DM	Mean rate of digestion ¹ , %/h
Corn	0	13.3	0.62	8.27
	6	7.5		
	24	4.9		
Soybean hulls	0	71.6	1.24	7.70
	6	38.8		
	24	25.9		
Corn gluten feed	0	33.9	0.93	3.95
	6	24.5		
	24	21.7		

¹The average rate of digestion was calculated using the NDF rate calculator (Van Amburgh et al., 2003) with fixed lag time of 3 hours.

The mean digestion rates in Table 3.2 represent the average of digestion rates of the NDF fractions of the various ingredients used in the experimental diets. Soybean hulls normally have a high rate of digestion for NDF but the high rate of NDF digestion for corn (8.27%/h) exhibited in Table 3.2 is highly unlikely, considering that with a fixed lag time of about 3 h, at the 6 h fermentation point, only about 3 h of fermentation could have taken place, and according to these data, 44% of the corn NDF digested in that 3 h time frame. Ideally, the starches and sugars should have been filtered out during the NDF analysis, but due to the limitations presented by the porosity of 1.5 microns, it is hypothesized that a portion of highly soluble cell contents such as sugars and starches remained present in the fraction that was designated NDF prior to the start of digestion. Thus, during the fermentation analysis, the extremely high rate of disappearance seen in the first hours of fermentation indicates that sugars and starches, which are characterized by rapid rates of digestion, were being fermented along with NDF. Moreover, corn generally contains approximately 9% NDF, and this analysis found the corn to have 13.3% NDF, also indicating that NDF was overestimated. If the NDF was overestimated in the corn, as seems apparent by the data, it was likely overestimated to some degree in the corn gluten feed, soy hulls and for the other NDF analyses of feed and feces as well.

In Table 3.3, it is apparent that ewe average daily DMI, average body weight and average daily gain increased significantly with the increase in diet FNDF from 15% to the average of 25% and 35% FNDF diets. However, the orthogonal contrast of 25% FNDF versus 35% FNDF diets only shows significant differences for average daily DMI ($P = 0.051$). The average ewe on the 15% FNDF diet lost weight during the experiment. The lower intake and weight loss may be due to metabolic problems in the rumen, which can be anticipated at high nonstructural carbohydrate levels

(NSC) relative to low FNDF in the diet. The 15% FNDF diet had 47% corn grain, which would feed the NSC bacteria in the rumen, producing more lactic acid, and thus increasing the acidity of the rumen.

Table 3.3 Ewe growth and dry matter intake

Diet	Initial wt, kg	Final wt, kg	Avg body wt, kg	Ewe ADG ¹ , g/d	Avg daily DMI ² , g	ADG/avg daily DMI
15% FNDF	57.7	56.7	57.2	-30	2,343	-0.028
25% FNDF	62.9	66.8	65.6	93	3,286	0.028
35% FNDF	61.8	66.2	65.8	105	3,871	0.028
SE	2.56	3.58	3.13	50.66	198.4	0.023
<i>P</i> value: 15 vs 25% & 35%	0.158	0.038	0.040	0.052	<0.001	0.059
<i>P</i> value: 25 vs 35%	0.765	0.905	0.967	0.870	0.051	0.997

¹ Average weekly change in body weight divided by 7 days

² Weekly intakes divided by 7 days

Average lamb final weights and average daily gains were significantly different between 25% FNDF and 35% FNDF diets but there was no significant difference between the 15% FNDF diet mean and the average of the 25% and 35% FNDF diet means (Table 3.4). While ewes fed the 35% FNDF diet had significantly higher DMI ($P = 0.051$) than ewes fed the 25% FNDF diet, their body weights ($P = 0.967$) and gains ($P = 0.870$) were not significantly higher than those of the ewes fed 25% FNDF (Table 3.3); meanwhile, lambs of ewes fed 35% FNDF had significantly higher final weights ($P = 0.013$) and average daily gains ($P = 0.007$). Therefore, the extra feed intake by ewes fed the 35% FNDF diet was apparently used for milk, which increased the growth of their lambs, although the lambs did occasionally steal feed

from the ewes' feed bins, so some of the attributed increased DMI to the ewes on higher FNDF diets may be in part, attributed to lamb DMI as well. No significant effects of diet were found for the number of lambs that finished the experiment, but a nonsignificant trend was evident for fewer lambs from ewes fed the 15% FNDF diet finishing the study (Table 3.4).

Table 3.4 Lamb growth for lambs that completed the experiment

Diet	Initial wt ¹ , kg	Final wt, kg	ADG for avg lamb ² , g	NOL last week ³
15% FNDF	4.3	11.6	174	1.7
25% FNDF	4.4	11.4	167	2.3
35% FNDF	4.6	14.2	229	2.4
SE	0.2867	0.8898	17.98	0.316
<i>P</i> value: 15 vs 25% & 35%	0.628	0.254	0.246	0.114
<i>P</i> value: 25 vs 35%	0.562	0.013	0.007	0.753

¹Average initial weight of only the lambs that completed the six week experiment.

²Calculated as (average lamb weight at week 6 – average lamb weight at week 0)/42 days

³Each treatment began with five sets of triplets and two sets of twins. Thus, the average number of lambs for each treatment at week 1 was 2.714.

No significant difference was found but a noticeable nonsignificant trend of increased average lamb gain per ewe with increase in diet FNDF occurred (Table 3.5). An increase in creep consumption by lambs was observed with increased dietary FNDF although it was only significantly different in the contrast between the 15% FNDF diet mean and the average of the 25% and 35% FNDF diet means, which reflects the lower average number of lambs for ewes fed the 15% FNDF diet. There was no apparent trend of lamb hay intake among diets. Total pen DMI increased

notably ($P < 0.001$, Table 3.5) with increased FNDF in the diet. There was an associated increase for total pen gain, but it was only significantly different for the first orthogonal contrast. The increased feed intake and corresponding gains could be attributed to the increased level of FNDF in the diets. There was no significant difference among diets in efficiency of gain (Table 3.5).

Table 3.5 Total ewe and lamb DMI and gain

Diet	Initial lamb wt, kg	Avg lamb gain per ewe ¹ , kg	Creep intake ² , kg DM	Hay intake ³ , kg DM	Total pen DMI ⁴ , kg	Total pen gain ⁵ , kg	Total pen gain/DMI, kg
15% FNDF	4.2	5.2	3.3	1.8	106	15.3	0.127
25% FNDF	4.3	6.6	6.3	1.5	144	20.9	0.143
35% FNDF	4.3	8.9	9.6	1.7	172	28.0	0.161
SE	0.229	1.151	1.445	0.327	9.650	3.715	0.0237
<i>P</i> value:							
15% vs 25% & 35%	0.675	0.082	0.018	0.693	<0.001	0.054	0.385
<i>P</i> value:							
25% vs 35%	0.936	0.187	0.124	0.773	0.040	0.164	0.578

¹Determined by the difference between each lamb's final weight and initial weight. Takes into account lambs removed from trial and lambs that died.

²Using percent dry matter calculated from the feeds; 91% dry matter for 15% FNDF, 90% dry matter for 25% FNDF and 90.5% dry matter for 35% FNDF to calculate the total creep consumed by an average lamb on that treatment.

³Using 90% dry matter for the hay to calculate total hay consumption of an average lamb on that treatment.

⁴The sum of ewe total DMI for 6 weeks and lamb total creep and hay DMI for 6 weeks, excluding data from the ewe and lambs in pen 4, which were on the experiment for 5 weeks.

⁵Sum of every lamb's total change in body weight and ewe total change in body weight, excluding data from the ewe and lambs in pen 4, which were on the experiment for 5 weeks.

Table 3.6a Calculation of NDF components for 15% FNDF diet using discount factors for level of feed intake

Item	Corn gluten feed	Corn	Soy hulls
(1) Digestible DM, %	83	87	80
(2) Van Soest (1992) discount per unit of maintenance above maintenance ¹ , %	13.5	3.3	18
(3) Units of maintenance for 15% FNDF diet ²	2.29	2.29	2.29
(4) Discount, % units [{(3)-1} x (2) x (1)/100]	14.45	3.70	18.58
(5) New digestible DM, % [(1)-(4)]	68.55	83.30	61.42
(6) Indigestible DM, %[100-(5)]	31.45	16.70	38.58
(7) Fecal metabolic losses, % of intake	10	10	10
(8) NDF, %	31.4	9	67
(9) Indigestible NDF, % [(6)-(7)]	21.45	6.70	28.58
(10) FNDF ³ , % [(8)-(9)]	9.95	2.30	38.42

¹(Van Soest et al., 1992).

² The NRC (2007) estimated DMI for 60 kg ewes at maintenance is 1.05 kg. (3) represents the units above this level for average feed intake while on the experiment. Average feed intake was 2.4 kg/d for ewes fed the 15% FNDF diet.

³Assuming tabular value of NDF.

Table 3.6b Calculation of NDF components for 25% FNDF diet using discount factors for level of feed intake

Item	Corn gluten feed	Corn	Soy hulls
(1) Digestible DM, %	83	87	80
(2) Van Soest (1992) discount per unit of maintenance above maintenance ¹ , %	13.5	3.3	18
(3) Units of maintenance for 25% FNDF diet ²	3.14	3.14	3.14
(4) Discount, % units [{(3)-1} x (2) x (1)/100]	23.98	6.14	30.82
(5) New digestible DM, % [(1)-(4)]	59.02	80.86	49.18
(6) Indigestible DM, %[100-(5)]	40.98	19.14	50.82
(7) Fecal metabolic losses, % of intake	10	10	10
(8) NDF, %	31.4	9	67
(9) Indigestible NDF, % [(6)-(7)]	30.98	9.14	40.82
(10) FNDF ³ , % [(8)-(9)]	0.42	0	26.18

¹(Van Soest et al., 1992).

² The NRC (2007) estimated DMI for 60 kg ewes at maintenance is 1.05 kg. (3) represents the units above this maintenance level for average feed intake while on the experiment. Average feed intake was 3.3 kg/d for ewes fed the 25% FNDF diet.

³Assuming tabular value of NDF.

Table 3.6c Calculation of NDF components for 35% FNDF diet using discount factors for level of feed intake

Item	Corn gluten feed	Corn	Soy hulls
(1) Digestible DM, %	83	87	80
(2) Van Soest (1992) discount per unit of maintenance above maintenance ¹ , %	13.5	3.3	18
(3) Units of maintenance for 35% FNDF diet ²	3.71	3.71	3.71
(4) Discount, % units [$\{(3)-1\} \times (2) \times (1)/100$]	30.37	7.78	39.02
(5) New digestible DM, % [(1)-(4)]	52.63	79.22	40.98
(6) Indigestible DM, % [100-(5)]	47.37	20.78	59.02
(7) Fecal metabolic losses, % of intake	10	10	10
(8) NDF, %	31.4	9	67
(9) Indigestible NDF, % [(6)-(7)]	37.37	10.78	49.02
(10) FNDF, ³ % [(8) – (9)]	0	0	17.98

¹(Van Soest et al., 1992).

² The NRC (2007) estimated DMI for 60 kg ewes at maintenance is 1.05 kg. (3) represents the units above this maintenance level for average feed intake while on the experiment. Average feed intake was 3.9 kg/d for ewes fed the 35% FNDF diet.

³ Assuming tabular value of NDF.

Table 3.7 Diet NDF components

Item	15% FNDF	25% FNDF	35% FNDF	SE	<i>P</i> value:	<i>P</i>
					15 vs 25% & 35%	value: 25 vs 35%
Digestion trial values						
Laboratory NDF, % of diet DM	22.8	32.4	41.5	0.11	<0.001	<0.001
Digestible NDF, % of NDF	31.9	35.8	38.8	3.07	0.166	0.507
FNDF, % of diet DM	7.3	11.6	16.1	1.03	<0.001	0.007
Indigestible NDF, % of diet DM	15.6	20.8	25.4	1.02	<0.001	0.005
Digestibility, % of DM	67.2	60.5	55.3	1.42	<0.001	0.019
Calculated values based on Van Soest discounts for level of feed intake						
NDF, % of diet DM	19.2	30.4	41.2			
Digestible NDF, % of NDF	30.4	18.3	17.5			
FNDF, % of diet DM	5.83	5.56	7.21			
Indigestible NDF, % of diet DM	13.3	24.9	36.7			
Digestibility, % of DM	70.4	59.4	48.0			

The top portion of Table 3.7 depicts the analyzed values for NDF from the digestion trial and the corresponding values for digestible NDF, FNDF, INDF and digestibility based upon the chromic-oxide determined digestibilities in this experiment. A discrepancy between the FNDF content originally formulated for (see Table 2.1.1) and the FNDF content as analyzed (Table 3.7) exists: the 15% FNDF diet actually contained 7.3% FNDF, the 25% FNDF diet contained 11.6% FNDF and the 35% FNDF diet contained 16.1% FNDF; all diets exhibited much higher INDF levels than expected. The expected NDF values in Table 2.1.1 (19.4%, 30.5%, and 41.1%) did not differ much from the digestion trial values of NDF in Table 3.7 (22.8%, 32.4%, and 41.5%). The lower observed FNDF levels (Table 3.7) compared to the 1X maintenance values for the designed experiment (Table 2.1.1) may be attributed to the

increased levels of intake and the corresponding depressions in digestibility that occurred as a result.

In the bottom portion of Table 3.7, calculated values are shown for NDF, digestible NDF, FNDF, INDF and digestibility using Van Soest's discount factors to account for the lower levels of digestibility associated with increased levels of intake above maintenance (Van Soest et al., 1992). The basis of these discount factors is that digestibility will decrease when feed intake surpasses that which is needed for maintenance, due partially to faster rate of passage from the rumen and overall loss of potentially digestible NDF (Van Soest et al., 1992). Small particles of concentrates are lost more easily because they are smaller, heavier and less likely to be ruminated than forage, which is lighter and larger (Van Soest et al., 1992).

The intake levels for the 15, 25 and 35% FNDF diets were, respectively, 2.3, 3.3 and 3.9 kg average daily DMI (Table 3.3). The 2007 NRC expected intake level at maintenance is 1.05 kg per day. Therefore, in Tables 3.6a through 3.6c, the units above maintenance intake for each diet were calculated and multiplied by the Van Soest discount factor associated with the corresponding feed ingredient. For instance, soy hulls have a discount of 13.5, which means that for each unit of maintenance above maintenance DMI, digestibility will decrease by 13.5%.

INDF was determined as 100 minus the discounted Digestible Dry Matter (DDM) minus the estimated fecal metabolic losses of 10%. FNDF was then determined by subtracting the INDF value from the amount of NDF in the given feed ingredient. FNDF for each feed ingredient was multiplied by the proportion of the feed ingredient in the diet and summed for all ingredients to arrive at the discounted FNDF level, reported in Table 3.7. The expected digestibilities (Table 2.1.1) for all diets at 1X maintenance were 78.4 to 79.3%. The observed digestibilities for 15, 25, and 35% FNDF diets were 67.2, 60.5, and 55.3% (top portion of Table 3.7).

Discounted digestibilities were 70.4, 59.4 and 48.0% (bottom portion of Table 3.7). The 35% FNDF diet appears to be more digestible than expected with the discounts given the high amount of average feed intake observed for ewes fed this diet (average daily DMI was 3.9 kg).

The Van Soest discounts do not account for the experimental differences in digestible NDF (the percentage of NDF that is digestible) and FNDF (digestible NDF as a percentage of the diet). Moreover, the digestion trial values for digestible NDF in Table 3.7 increase from 15% FNDF to 35% FNDF levels (31.9% to 38.8%) although the digestibility of these diets is decreased (67.2% to 55.3%). This conundrum might be explained by the overestimation of NDF in the feces relative to the feeds due to the 1.5 micron porosity of filters used because digestible NDF and FNDF values were determined using NDF values from the feed and feces, which we believe to be overestimated due to the limitations associated with such small micron size of filter porosity. The porosity difference may have caused greater error in the 15% FNDF diet because there was less NDF in this diet.

INDF in most literature is defined as lignin x 2.4, to account for lignin bound proteins and structures. INDF is referred to as the portion of NDF that remains completely unavailable to rumen microbes, or unavailable NDF. If we were to compare the values generated by that definition of INDF to the INDF values calculated from lignin values in Table 3.2 or the INDF values at 1X maintenance in Table 2.1.1, there would be drastic overestimation of INDF in the current diet formulations. INDF is shown in Table 3.7 to be 15.6, 20.8 and 25.4% of the diet for 15, 25, and 35% FNDF diets. If INDF were defined as lignin x 2.4, INDF would be 1.7, 2, and 2.3% for the respective diets, using the lignin values from Table 3.2. This would also increase the values for FNDF if $\text{NDF} - \text{INDF} = \text{FNDF}$. However, the value of 2.4 applies to forages and further research is required to locate a specific

multiplier that accurately describes lignin-bound unavailable proteins and substances for soy hulls, corn gluten feed and corn. Therefore, further research may be necessary to chemically characterize the INDF and FNDF components referenced in this experiment.

Based on average daily intakes and the percentages of INDF in the diets, the ewes fed the 15% FNDF diet consumed 0.36 kg INDF while those fed the 25% FNDF diet consumed 0.69 kg INDF and those fed the 35% FNDF diet consumed 0.99 kg INDF. Thus, as FNDF increased from the 15% FNDF diet to the 35% FNDF diet, INDF intake also increased. This positive correlation suggests that feed intakes of the ewes in this experiment were not limited by NDF or INDF levels in the diets.

Table 3.8 Milk yield and composition¹

	Milk yield, ² g	Milk fat, %	True protein %	SCC ³ x 1000	MUN, mg/dL	CP, ⁴ %	Lactose, %	Total solids, %
15% FNDF	2,663	10.2	5.37	7,312	23.2	5.03	4.58	20.8
25% FNDF	3,120	6.38	4.23	4,812	21.6	4.43	5.42	16.9
35% FNDF	3,589	7.14	4.37	1,253	26.2	4.56	5.44	17.7
SE	563.1	1.002	0.6233	2,704	1.523	0.5580	0.4010	1.177
<i>P</i> value: 15% vs 25%,35 %	0.329	0.009	0.154	0.213	0.694	0.445	0.082	0.018
<i>P</i> value: 25% vs 35%	0.564	0.583	0.870	0.364	0.034	0.863	0.981	0.609

¹ Composition data represent all ewes except ewe 14 on 25% FNDF because her milk was too mastitic to run through analyses.

² These numbers were extrapolated from 3 h milking data to represent 24 h milk yield.

³ Somatic cell counts are due to the high incidences of mastitis and udder problems.

⁴ No crude protein value was available for ewe 13 fed the 15% FNDF diet because the fat content was too high for the machine that assesses crude protein.

Table 3.9 Milk yield and composition¹ in terms of yield

Item	Milk yield, ² g	Milk fat yield, g	True protein yield, g	Crude protein, yield, ³ g	Lactose yield, g	Total solids yield, g
15% FNDF	2,663	235	120	120	138	513
25% FNDF	3,120	211	147	148	182	561
35% FNDF	3,589	275	163	164	196	654
SE mean	563.1	58.63	28.33	28.34	35.59	119.8
<i>P</i> value:						
15% vs 25%,35%	0.329	0.911	0.363	0.289	0.231	0.502
<i>P</i> value:						
25% vs 35%	0.564	0.429	0.654	0.682	0.771	0.576

¹ Composition data represent all ewes except ewe 14 on 25% FNDF because her milk was too mastitic to run analyses on.

² These numbers were extrapolated from 3 hour milking data to represent 24 hour milk yield.

³ No crude protein value was available for ewe 13 on 15% FNDF because the fat content was too high for the machine that assesses crude protein.

No significant differences in milk composition or yield can be attributed to an increase in dietary FNDF (Tables 3.8 and 3.9). However, there was an insignificant trend for increased milk production and total solids with increase in dietary FNDF. The observed milk yields in this experiment were relatively high compared with typical milk yields for non-dairy commercial ewes, which average 45 to 68 kg in a 90 to 100 day lactation, or 0.45 to 0.76 kg/d of milk (Thomas, 1996). However, these milk yields were extrapolated to 24 h milk yields when in fact they were only measured for 3 h, so this may not be representative of 24 h milk yield. Nursing triplets will also cause high milk production. In addition, milk yield was measured around the time of peak lactation in the experiment.

When Cannas et al. studied the relationship between MUN levels and dietary protein levels (Cannas et al., 1998), they found an MUN level of 17.0 mg/dL associated with a dietary CP level of 16.6% and an MUN level of 22.3 mg/dL associated with dietary CP level of 18.8%. The diets for this experiment contained dietary CP levels around 17% but the MUN levels are all between 21.6 mg/dL and 26.2 mg/dL (Table 3.8) which seem higher than would be expected. Perhaps this can be attributed to the high levels of DMI, which resulted in higher protein intakes. Total solids are usually 17.5% of sheep milk (Pulina et al., 2002) so the 25% and 35% FNDF diets resulted in milk solids similar to normal composition. The fat content of ewe milk from the 15% FNDF diet may be higher than that of the 25% FNDF diet because there were higher incidences of udder infections at the time of milking in ewes on the 15% FNDF diet, causing the milk components to be more concentrated with very low yields. It is difficult to make solid conclusions about the effects of diet on the milk data, especially due to the confounding brought about by the incidence of mastitis and udder sores. The high SCC levels indicate the amount of udder infections since the upper threshold for SCC in uninfected mammary glands of sheep ranges from 260,000 to 1,580,000 cells/mL (McDougall et al., 2001).

Two hundred and ninety-two ewes lambled during the March lambing at the Cornell Sheep Farm. Of the 271 ewes that were not on the experiment, not one received treatment for a case of mastitis. In the experimental group of ewes, 9 out of 21, or 42.9% were treated for mastitis, and 90.5% were treated for an udder-related problem of mastitis, sores or soremouth (contagious ecthyma) (Table 3.10). There was one case of contagious ecthyma, a ewe on the 35% FNDF diet. Although 3 of the ewes were treated for mastitis prior to April 15 and with no apparent teat chewing, by the week of April 15, sores were apparent on 17 of the 21 ewes around the base of the

teat where the lambs' teeth reside during suckling. These udder health problems may have resulted from lamb boredom which led to the chewing on ewe teats, or decreased chemotactic response of neutrophils, leading to decreased immune response which can be caused by states of negative energy balance (Kimura et al., 2001), but no evidence exists to concretely explain the udder health issues.

Table 3.10 Occurrence of udder-related health problems and treatments

Diet	Number of ewes with sores ¹	Total number of ewes treated	Total number of treatments ²	NOL died	NOL Removed ³
15% FNDF	7	3	10	1	6
25% FNDF	6	3	18	0	3
35% FNDF	6	3	22	2	0
<i>P</i> value ⁴ :	0.949	1.000	0.106	0.368	0.050

¹A number of ewes developed teat sores of varying levels of severity while on the experiment.

²Treatments qualify as a 10 cc injection of Penicillin-G for mastitis.

³A lamb was removed if it was clear that the lamb or one of its siblings would not survive if left with the ewe. Removal of one lamb took place in order to allow more total milk per lamb in the pen.

⁴Determined by Chi-square analysis.

Chi-square analysis was performed. There was a trend for more mastitic treatments for increased levels of FNDF (Table 3.10). The total number of ewes treated for mastitis was equal among treatments, indicating that each diet was equally responsible or not responsible for causing the mastitis.

Five hundred and ten lambs were born to the 292 ewes that lambed in March 2006, with an average lambing percentage of 1.67 live lambs per ewe. The ewes selected for the experiment had an average lambing percentage of 2.71, although this was controlled through experimental selection for twins and triplets. Lamb death loss from 0 to 60 days in the flock for the March 2006 ewes was 22.6%, although this

seemed to be extremely high and in the March 2007 lambing, the lamb death loss from 0 to 60 days of age was only 11.2%. Lamb death loss in the experiment was 5.3% with no significant difference among diets. Lambs that were removed or died on the trial was 21.1% with a significant difference ($P < 0.050$) among treatments (Table 3.10). The 15% FNDF diet had a significant positive effect on lamb removal while the 35% FNDF diet did not appear to cause necessity of lamb removal. This reflects the higher milk production of ewes fed the 35% FNDF diet.

Table 3.11 Average blood metabolite levels¹

Item	BHBA, mg/dL	NEFA, meq/L	PUN, mg/dL	D-lactate, mg/dL	L-lactate, mg/dL
Diet					
15% FNDF	6.25	183	16.8	65.2	248
25% FNDF	6.43	125	19.1	65.0	228
35% FNDF	7.14	186	20.7	65.1	231
SE	0.50	25.6	0.72	0.53	14.90
<i>P</i> value:					
15% vs 25% & 35%	0.37	0.39	<0.001	0.79	0.33
<i>P</i> value:					
25% vs 35%	0.32	0.11	0.204	0.90	0.89
Effect of Week					
	7.83	430	16.2	66.9	199
	5.80	194	16.3	63.8	197
	6.91	129	19.0	64.6	204
	7.10	87.7	19.5	65.7	187
	5.94	86.7	18.7	64.5	159
	6.10	96.2	19.7	64.3	182
	6.56	129	22.6	65.9	522
SE	0.509	29.4	1.05	0.78	21.1
<i>P</i> value:	0.016	<0.001	<0.001	0.061	<0.001

¹There were no significant interaction effects.

In Table 3.11, average blood metabolite values are shown for Beta-hydroxybutyrate (BHBA), Non-Esterified Fatty Acid (NEFA), Plasma Urea Nitrogen (PUN), D-lactic acid and L-lactic acid weekly levels. There was a significant difference between PUNs for the first orthogonal contrast. Although there were no significant differences among diets for the other blood metabolite levels, the effect of week was significant for all weekly blood metabolite levels (Table 3.11). Compared to reference values for blood metabolites, BHBA levels were well under ketosis diagnosis, which is usually around 10.4 mg/dL and the level of PUNs indicate a high level of protein as they are typically less than 16 mg/dL in sheep.

Blood Metabolite Weekly Levels

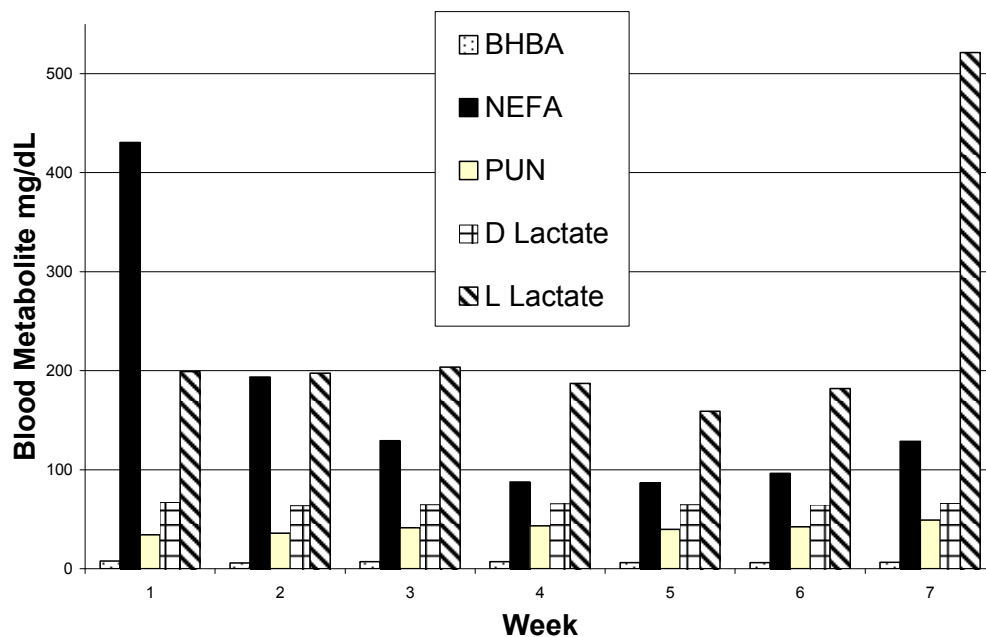


Figure 3.1 Beta-hydroxybutyrate, Non-Esterified Fatty Acid, Plasma Urea Nitrogen, D-lactic acid and L-lactic acid weekly levels in ewes fed 15, 25, and 35% FNDF diets.

The high NEFA concentration in the blood for the first measurement is likely due to the stress level the ewes endured the first week as they were chosen from their pens, moved down aisles for weighing, placed into the experimental pens, after which

blood was immediately drawn. The spike in L-lactic acid levels might be attributed to the udder problems present in the latter part of the study (Figure 3.1). It cannot be attributed to methods because blood was assayed by ewe and not by week; therefore, week 7 values were found consistently high on a variety of different microplates used for assays. Reparative tissues are very anaerobic and the energy needed for repair is largely taken from glucose metabolism to lactate followed by lactate diffusion from the damaged tissues back to the circulation to be made back into glucose by the liver (Cahill, 1986). Thus, the tissue repair in the udder around the time of the sixth week of the study may have caused the huge increase in circulating observed L-lactate levels.

4. CONCLUSIONS

Twenty-one ewes and their twin or triplet lambs during the March 2006 lambing season at the Cornell University Sheep Farm were used for a total of 6 weeks to evaluate the effects of increasing levels of FNDF (15, 25 and 35% of the diet) on DMI and production. The ewes exhibited significant increases ($P = 0.051$) in DMI with corresponding increases in FNDF level. Total ewe and lamb DMI and gain was also positively associated with increased levels of FNDF in the ewe's feed and the lamb creep feed. No solid conclusions were made from the milk data due to the inaccuracy of yields and compositions resulting from high incidences of udder diseases. There were no significant differences in blood metabolites as an effect of FNDF levels in the diets.

Expected and observed total diet dry matter digestibility differences were almost completely accounted for by Van Soest's digestibility discount factors for increased levels of intake but these discount factors did not account for the NDF related differences. Differences between formulated and analyzed values of FNDF in the diets may be due to the difference in porosity of the filters used for NDF analysis.

APPENDIX 1

1.1 Beta-hydroxybutyrate Assay

An adaptation of a Sigma Kit procedure #310-UV (St. Louis, MO) was used to perform the assay.

The following reagents were used per each 96-well flat-bottom plate.

1. 0.2 M TRIS buffer/NAD: Dissolve 4.844 g TRIS [hydroxymethyl] aminomethane in 180 mL double distilled water (ddH₂O) and adjust to pH of 9.0. Add ddH₂O to obtain 200 mL of solution and store at 4°C. On the day of the assay, mix 30 mg β-Nicotinamide adenine dinucleotide hydrate (NAD) (Sigma N-7004) in 15 mL of TRIS buffer.
2. Enzyme 3-HBDH: (Roche Diagnostics Corporation, from *Rhodopseudomonas spheroids*). On the day of the assay, centrifuge 90 μL of stock enzyme in a microcentrifuge tube (5000 rpm for 3 min), discard the supernatant, and resuspend pellet in 1.2 mL ddH₂O.
3. Standards: To make a 100 mg/dL stock solution, dissolve 242.307 mg DL-β-Hydroxybutyric acid sodium salt in 100 mL. Dilute the stock with ddH₂O to make aliquots containing 5, 10, 25, and 50 mg/dL, and store frozen.

Use the following procedure:

1. Set the plate reader temperature to 37°C.
2. Use 96-well flat-bottom plates.
3. Add to each well:
 - a. 5 μL sample/standard/water
 - b. 150 μL buffer/NAD
4. Mix in the plate reader and read the absorbance at 340 nm.
5. Add to each well 10 μL 3-HBDH enzyme.
6. Mix in the plate reader.

7. Incubate for 1 hour at 37°C.
8. Read the absorbance at 340 nm.

1.2 D-Lactic Acid and L-Lactic Acid Assay

After thawing, the plasma was centrifuged for 10 min at 4°C and 2500 x g in a Beckman Coulter Allegra 6R centrifuge. Supernatant (0.3 µL) was transferred to a microfuge tube for each sample. Next, the supernatant was neutralized with 6 M KOH (0.0525 µL) and vortexed. After 10 min of refrigeration, samples were centrifuged for 10 min using the same microcentrifuge at 4°C and 2500 x g. Three hundred µL of the supernatant was transferred into a 1.5 mL tube. The samples were frozen until used for assays.

The protocol for the L and D-lactic acid assay was adapted from Bryk-Lucy (2000).

The following reagents were used:

1. Buffer + NAD solution; Prepare fresh daily per plate the following:
 - a. 25 mL NAD, mg
 - b. 10 mL water
 - c. 5 mL Gly+hydrazine buffer

This will be sufficient for 100 slots.

2. Prepare the enzyme L(D)-LDH daily by diluting it to contain 600 U/ml. For the enzyme L-LDH, add 1 mL water to the bottle to yield 552.75 U/ml, sufficient for 4 plates.

3. Standards: Prepare L- and D- stock standards 40 mg/10 mL for assays. Prepare a 1:100 dilution of stock to yield 40 mg/dL (400 mg/L). Use serial dilution of 1:100 to prepare 20, 10, 5, 2.5 and 1.25 mg/dL.

Use the following procedure:

1. Set the plate reader to 37°C.

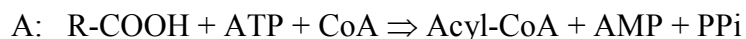
2. Pipet the following into a 96-well flat plate:
 - a. 28 μL sample/standard/blank
 - b. 84 μL buffer+NAD
3. Place the plate into the plate reader. Let the temperature equilibrate for approximately 3 min and read the absorbance at 340 nm.
4. Add to each well 5.6 μL L(D)-LDH.
5. Mix in the plate reader and incubate for 45 min.
6. Read the absorbance at 340 nm.

Do quadruplicates of sample/standard/blank and include a standard curve for each plate.

1.3 Non-esterified fatty acids (NEFA) Assay

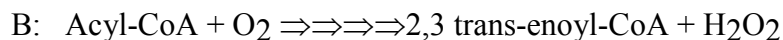
Enzymatic analysis using NEFA-C Reagents from WAKO (Richmond, VA) was used to perform the assay with the following adaptations. Reagents A and B were further diluted and used in proportion to each other. The principles of this assay are based upon these reactions:

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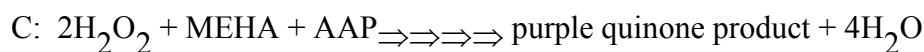
FFA is activated to CoA ester by acyl CoA synthetase.

ACOD



Acyl-CoA is oxidized by Acyl CoA oxidase (ACOD) to produce hydrogen peroxide.

POD



Hydrogen peroxide is acted on by peroxidase (POD) in the presence of 3-methyl-N-ethyl-N-β-hydroxyethyl-aniline (MEHA) and 4-aminoantipyrine (AAP) to form product with a purple color which is read at 550 nm.

The following reagents were used:

1. Color Reagent "A"; total volume will be 23.3 mL; enough for about 330 wells.
2. Diluent for Color Reagent A.
3. Color Reagent "B"; total volume will be 53.3 mL; enough for about 330 wells.
4. Diluent for Color Reagent B.
5. 0.5 M phosphate buffer, pH 6.9. Dissolve the following into less than 100 mL of ddH₂O and adjust to pH 6.9, bringing the final volume to 100 mL:
 - a. 0.45 g Na₂PO₄ 7H₂O
 - b. 0.46 g NaH₂PO₄ H₂O
6. Standards:

0	1000 μL of ddH ₂ O
125	875 μL of ddH ₂ O + 125 μL of μEg/L NEFA standard provided in kit.
250	750 μL of ddH ₂ O + 250 μL of μEg/L NEFA
500	500 μL of ddH ₂ O + 500 μL of μEg/L NEFA
1000	1000 μL of μEg/L NEFA

The following procedure was used:

1. Add 10 mL of Diluent A to Reagent A vial; mix and add 13.3 mL of 0.05 M phosphate buffer.
2. Add 20 mL of Diluent B to Reagent B vial; mix and add 33.3 mL of 0.05 M phosphate buffer.
3. Pipet into flat bottom 96-well plates:
 - a. 5 μL serum, plasma, standard or H₂O
 - b. 70 μL Reagent A

4. Mix and incubate at 37 °C for 20 min.
5. Add 160 µl Reagent B to each well.
5. Mix and incubate at 37°C for 20 min.
6. Read the plate at 550 nm.

1.4 Plasma urea nitrogen (PUN) assay

PUN's were performed in the laboratory using a method based on Sigma Kit No. 640 (St. Louis, MO).

The following reagents were used:

1. Phenol nitroprusside solution (Sigma P6994)
2. Alkaline hypochlorite solution (Sigma A1727)
3. Urease Type IV buffer reagent from jack beans (Sigma U4002)

-Add 30 mL of water to the vial.

4. Urea N standard solution
 - a. 100 mg/L urea N standard solution is prepared.
 - b. Dilute the standards from 0 to 100; they are stable for about one day

The following procedure was used:

1. Add 250 µL of urease solution and 20 µL sample/standard/blank into 16x100 test tubes.
2. Vortex the tubes gently.
3. Allow to stand 20 min at room temperature (urea hydrolyzes to NH₃)
4. Add in order, vortexing after each addition:
 - a. 500 µL phenol nitroprusside
 - b. 500 µL alkaline hypochlorite
 - c. 5 mL double distilled water
5. Incubate at least 1 h at room temperature; color will be stable for several hours.

6. Read the absorbance.
 - a. Set the wavelength at 570 nm.
 - b. Zero the spectrometer with double distilled water.
 - c. Read duplications of the blank first and if the duplication seems far apart, pool tubes, vortex and rezero the spectrometer.
 - d. Vortex all the tubes prior to reading; color tends to stratify in test tube.

Determine the results from a regression curve of the standards using the results from the dilution set described above.

APPENDIX 2

Feed Data

Pen	EweID	Diet	Wk1 Daily DMI, kg	Wk2 Daily DMI, kg	Wk3 Daily DMI, kg	Wk4 Daily DMI, kg	Wk5 Daily DMI, kg	Wk6 Daily DMI, kg	Lamb creep feed DMI, kg	Lamb hay DMI, kg
3	W658	15%	2.5	2.7	1.7	1.3	2.4	2.6	1.60	2.00
4	CXB3690	15%	2.9	2.7	1.3	1.0	0.7		2.40	0.60
8	CXB4238	15%	2.1	2.4	2.4	2.6	2.7	2.4	5.48	2.00
9	F2808	15%	2.5	3.0	3.0	3.1	3.2	3.2	4.50	2.00
11	CXB5045	15%	2.0	1.9	2.1	1.6	1.2	1.4	4.00	3.00
13	CXB3432	15%	2.2	1.9	1.3	2.2	1.9	2.2	0.00	0.25
20	P2313	15%	3.3	3.6	3.3	3.6	3.4	3.2	5.19	2.46
1	Pu286	25%	3.1	3.5	3.8	3.6	4.2	4.0	6.35	2.50
6	CXB3496	25%	2.7	2.9	2.7	2.9	2.3	1.9	4.93	0.80
10	P2309	25%	3.3	3.9	4.6	2.1	2.6	1.4	5.43	2.43
14	F3011	25%	3.3	2.9	2.8	4.0	3.2	3.2	7.99	1.00
16	CXB2979	25%	2.8	3.3	3.5	3.6	3.4	3.1	6.56	1.98
18	F2841	25%	3.6	4.1	4.4	4.3	3.8	3.3	8.05	1.00
19	CXB2791	25%	2.9	2.6	3.3	3.5	3.6	3.7	4.60	1.00
2	F2995	35%	3.1	3.4	4.1	4.0	4.3	4.0	7.40	0.99
5	CXB3429	35%	3.5	3.5	3.8	3.8	3.6	1.4	2.00	1.00
7	CXB3064	35%	3.7	4.1	4.2	4.3	4.3	2.9	14.99	1.50
12	P2143	35%	3.4	3.9	4.4	4.4	4.5	5.0	2.00	1.50
15	CXB4513	35%	2.4	2.9	3.5	3.2	3.3	3.7	9.65	3.50
17	CXB3202	35%	4.0	3.9	4.4	4.5	4.5	4.3	17.49	1.67
21	CXB2112	35%	3.8	4.8	4.6	4.1	4.6	4.3	13.48	1.50

APPENDIX 3

Digestion Data

Pen	EweID	Diet	Sample date	Lab feed Cr, mg/L	Lab feed sample volume, L	Lab feed sample wt, g	Lab feed DM, %	Lab feces Cr, mg/L	Lab feces sample volume, L	Lab feces sample wt, g	Lab feces DM, %	Feed NDF, % DM	Feces NDF, % DM
3	W658	15%	5/4/2006	6.997	0.200	0.230	91.000	22.271	0.200	0.210	96.000	0.2274	0.5052
4	CXB3690	15%	5/9/2006	6.997	0.200	0.230	91.000	21.353	0.200	0.245	95.000	0.2274	0.4450
8	CXB4238	15%	5/4/2006	6.997	0.200	0.230	91.000	23.087	0.200	0.230	96.000	0.2274	0.5342
9	F2808	15%	5/4/2006	6.997	0.200	0.230	91.000	22.075	0.200	0.230	95.000	0.2274	0.5156
11	CXB5045	15%	5/4/2006	6.997	0.200	0.230	91.000	20.351	0.200	0.200	95.000	0.2274	0.4318
13	CXB3432	15%	5/4/2006	6.997	0.200	0.230	91.000	20.906	0.200	0.220	95.000	0.2274	0.4225
20	P2313	15%	4/25/2006	5.699	0.200	0.205	91.000	18.3675	0.200	0.210	96.000	0.2355	0.4694
1	Pu286	25%	5/4/2006	9.2875	0.200	0.205	90.000	21.951	0.200	0.205	96.000	0.3242	0.5345
6	CXB3496	25%	5/4/2006	9.2875	0.200	0.205	90.000	26.216	0.200	0.200	95.000	0.3242	0.5081
10	P2309	25%	4/25/2006	8.266	0.200	0.220	92.000	25.867	0.200	0.235	97.000	0.3245	0.4738
14	F3011	25%	4/25/2006	8.266	0.200	0.220	92.000	24.507	0.200	0.215	97.000	0.3245	0.5026
16	CXB2979	25%	4/25/2006	8.266	0.200	0.220	92.000	19.965	0.200	0.200	98.000	0.3245	0.6285
18	F2841	25%	5/4/2006	9.2875	0.200	0.205	90.000	26.066	0.200	0.215	95.000	0.3242	0.4981
19	CXB2791	25%	5/4/2006	9.2875	0.200	0.205	90.000	23.770	0.200	0.220	96.000	0.3242	0.5284
2	F2995	35%	5/4/2006	6.8575	0.200	0.200	90.000	21.445	0.200	0.230	96.000	0.4128	0.5754
5	CXB3429	35%	5/4/2006	6.8575	0.200	0.200	90.000	21.095	0.200	0.225	96.000	0.4128	0.5633
7	CXB3064	35%	5/4/2006	6.8575	0.200	0.200	90.000	14.867	0.200	0.205	95.000	0.4128	0.6065
12	P2143	35%	5/4/2006	6.8575	0.200	0.200	90.000	17.249	0.200	0.200	95.000	0.4128	0.5840
15	CXB4513	35%	4/25/2006	6.3185	0.200	0.220	88.000	14.108	0.200	0.215	98.000	0.4214	0.5329
17	CXB3202	35%	4/25/2006	6.3185	0.200	0.220	88.000	14.845	0.200	0.215	97.000	0.4214	0.5411
21	CXB2112	35%	5/4/2006	6.8575	0.200	0.200	90.000	17.715	0.200	0.235	95.000	0.4128	0.5794

APPENDIX 4

Ewe Weight Data

PenNo	EweID	Diet	Wk0 Wt, kg	Wk1 Wt, kg	Wk2 Wt, kg	Wk3 Wt, kg	Wk4 Wt, kg	Wk5 Wt, kg	Wk6 Wt, kg	AVG Wt, kg
3	W658	15%	56.4	58.6	60.9	54.1	54.5	62.7	65.3	58.9
4	CXB3690	15%	52.7	55.0	54.5	47.5	46.8	45.0		50.3
8	CXB4238	15%	55.0	54.1	56.8	57.3	59.2	58.2	56.8	56.8
9	F2808	15%	59.1	58.2	63.2	62.3	61.0	60.0	61.4	60.7
11	CXB5045	15%	53.2	50.0	48.2	47.3	43.6	40.9	40.4	46.2
13	CXB3432	15%	57.3	55.0	49.5	52.3	54.8	52.7	53.0	53.5
20	P2313	15%	70.5	70.9	72.7	76.8	77.3	74.3	75.0	73.9
1	Pu286	25%	64.5	65.1	69.1	70.5	71.6	72.7	71.9	69.3
6	CXB3496	25%	58.2	59.5	61.8	62.3	60.9	62.7	62.5	61.1
10	P2309	25%	68.2	66.4	73.6	75.5	71.8	75.0	66.8	71.0
14	F3011	25%	54.1	50.9	48.5	54.5	57.7	57.9	58.2	54.5
16	CXB2979	25%	54.5	54.5	56.4	59.5	62.7	61.5	61.4	58.6
18	F2841	25%	66.8	63.2	70.5	71.4	72.1	69.1	67.5	68.7
19	CXB2791	25%	74.1	72.1	73.2	76.8	78.0	77.7	79.5	75.9
2	F2995	35%	70.0	73.6	77.3	80.0	79.1	81.4	82.2	77.7
5	CXB3429	35%	57.7	60.5	65.5	67.7	66.1	65.0	59.6	63.2
7	CXB3064	35%	60.9	63.6	67.3	66.4	66.2	65.0	59.2	64.1
12	P2143	35%	65.5	65.0	73.2	70.0	73.3	66.8	66.8	68.7
15	CXB4513	35%	49.5	47.3	46.8	50.9	47.7	54.2	57.3	50.5
17	CXB3202	35%	63.6	65.5	66.4	70.5	73.6	72.5	71.4	69.1
21	CXB2112	35%	65.5	64.5	69.1	70.5	64.4	68.6	67.0	67.1

APPENDIX 5

Ewe Body Condition Score Data

Pen	EweID	Diet	Wk0 BCS	Wk1 BCS	Wk2 BCS	Wk3 BCS	Wk4 BCS	Wk5 BCS	Wk6 BCS
3	W658	15%			2.5	1.5	2	2	2
4	CXB3690	15%		3	2	2.5	1.5	1.5	
8	CXB4238	15%			3	3	3	3.5	3.5
9	F2808	15%			2.5	3	3	2.5	2.5
11	CXB5045	15%			2.5	2	1.5	1.5	2
13	CXB3432	15%			2.5	1.5	1.5	2	1.5
20	P2313	15%				4	4	4	4
1	Pu286	25%			3.5	3.5	3.5	3.5	3.5
6	CXB3496	25%			3	2.5	2	2	2
10	P2309	25%				3	3.5	2.5	
14	F3011	25%				1.5	2	2	2
16	CXB2979	25%				2.5	2.5	2.5	2.5
18	F2841	25%			2	2.5	2	2	2.5
19	CXB2791	25%			3.5	3.5	3.5	4	3.5
2	F2995	35%			3	3	3	3	2.5
5	CXB3429	35%			3	2.5	2.5	2	2
7	CXB3064	35%			3	3	2.5	3	2.5
12	P2143	35%			2	2.5	2.5	3	3
15	CXB4513	35%				2	1.5	1.5	
17	CXB3202	35%				2.5	2.5	2.5	3
21	CXB2112	35%			3.5	3.5	3	3.5	3

APPENDIX 6

Milk Data

PenNo	EweID	Diet	Yield, kg	24 hour yield, g	Fat, %	True protein, %	SCC x 1,000	Crude protein, %	Lactose, %	Total solids, %	MUN, mg/dL
3	W658	15%	0.23	1818	8.05	3.88	1114	4.03	5.59	18.24	21.5
4	CXB3690	15%	0.23	1818	14.15	9.65	21903	9.8	3.01	27.8	*
8	CXB4238	15%	0.55	4364	7.42	3.81	279	3.97	5.42	17.4	26.2
9	F2808	15%	0.41	3273	7.41	3.95	2913	4.12	5.55	17.66	21.0
11	CXB5045	15%	0.27	2182	9.05	4.15	2723	4.3	5.17	19.06	30.5
13	CXB3432	15%	0.05	364	16.12	8.29	21860		1.59	26.31	16.9
20	P2313	15%	0.59	4727	8.905	3.825	393.5	3.935	5.725	19.12	23.1
1	Pu286	25%	0.36	2909	7.01	4.55	691	4.77	5.41	17.78	27.1
6	CXB3496	25%	0.27	2182	7.94	4.16	3632	4.34	5.05	17.91	19.4
10	P2309	25%	0.32	2545	4.285	4.035	153.5	4.26	5.6	14.835	24.6
14	F3011	25%	0.23	1818	*	*	20709	*	*	*	13.8
16	CXB2979	25%	0.55	4364	4.745	3.635	586	3.83	5.77	15.02	21.2
18	F2841	25%	0.50	4000	6.71	5.04	4090	5.29	5.33	17.94	22.5
19	CXB2791	25%	0.50	4000	7.56	3.93	3824	4.09	5.38	17.63	22.8
2	F2995	35%	0.23	1818	7.13	4.42	2253	4.63	4.96	17.31	23.2
5	CXB3429	35%	0.27	2182	6.06	5.02	2986	5.3	5.32	17.29	26.2
7	CXB3064	35%	0.27	2182	5.48	4.01	426	4.23	5.74	16.08	28.0
12	P2143	35%	0.36	2909	6.25	4.09	330	4.3	5.53	16.69	27.9
15	CXB4513	35%	0.55	4364	8.52	3.96	2313	4.085	5.475	18.36	26.1
17	CXB3202	35%	0.82	6545	10.28	4.8	312	4.92	5.44	21.16	24.6
21	CXB2112	35%	0.64	5091	6.25	4.26	153	4.48	5.59	16.93	27.6

APPENDIX 7

Lamb Weight Data

PenNo	EweID	Diet	Lamb ID	Wk0	Wk1	Wk2	Wk3	Wk4	Wk5	Wk6	Early final wt, kg	Days on trial
				wt, kg	wt, kg	wt, kg	wt, kg	wt, kg	wt, kg	wt, kg		
3	W658	15%	5249	5.4	6.6	6.6	6.3				6.3	21
3	W658	15%	5250	4.4	5.4	6.2	5.8				5.8	21
3	W658	15%	5251	4.1	5.1	6.2	6.4	6	7.9	9.4		42
4	CXB3690	15%	5378	4.2	5.3	6.2	4.5				4.5	21
4	CXB3690	15%	5379	4.5	5.9	7	5.9	5.3			5.3	28
4	CXB3690	15%	5381	3.7	4.1	4.9					4.9	14
8	CXB4238	15%	5227	5	5.8	7.3	8.4	9.8	12	12.8		42
8	CXB4238	15%	5228	4.1	5.2	6.4	7.5	9.3	11.3	12.5		42
9	F2808	15%	5288	4.4	5.8	6.8	7.9	10.1	12.5	14.5		42
9	F2808	15%	5289	3.6	5	5.4	6.1	7.4	8.8	10.1		42
9	F2808	15%	5290	4.6	6	7.1	8	9.4	10.4	11		42
11	CXB5045	15%	5231	5.6	8	9.5	10.4	11	11.5	12.7		42
11	CXB5045	15%	5232	5.2	6.7	7.6	8.1	9	10.5	11.5		42
13	CXB3432	15%	5254	2.7	3.1	2.9					2.9	14
13	CXB3432	15%	5255	3.6	4.6	4.8					4.8	14
13	CXB3432	15%	5256	4.8	5.9	6.7	6.6	7.6	8	8.9		42
20	P2313	15%	5154	3.9	5.9	6.8	7.1	8	9.7	11.4		42
20	P2313	15%	5155	3.1	5.1	6.3	7	8.1	9.7	11.6		42
20	P2313	15%	5156	3.2	4.3	6.2	7.3	8.4	10.5	12.6		42
1	Pu286	25%	5221	4.2	5.2	6.6	7.9	9.4	11.4	12.5		42
1	Pu286	25%	5222	5.6	7.6	9.1	10.8	13.1	15.3	17.4		42
6	CXB3496	25%	5296	4.1	4.1	4.9	5.9	5	4.1		4.1	35

6	CXB3496	25%	5297	3.9	4.5	5.5	5.8	6.2	6	6.8	42
6	CXB3496	25%	5298	3.1	4.1	4.9	5.5	6.2	6.5	7.3	42
10	P2309	25%	5141	3.5	4.9	6	6.4	5.4	7.5	8.3	42
10	P2309	25%	5142	2.5	3.5	4.7	4.8	4.6	5.8	6	42
10	P2309	25%	5143	4.2	5.7	7.4	9.2				21
14	F3011	25%	5151	5.2	6.5	6.5	7.5	9.7	11.7	13	42
14	F3011	25%	5152	4.3	6	6.6	7.3	8.8	10.1	10.1	42
14	F3011	25%	5153	5	5.9	6.2	6	6.5	6.6	7.7	42
16	CXB2979	25%	5052	3.3	3.9	4.3	4.8	6.7	8.8	9.5	42
16	CXB2979	25%	5053	3.9	4.7	5.6	5.9	7.8	9.9	11.3	42
16	CXB2979	25%	5054	5.1	7	8.6	10.1	11	9.4	11	42
18	F2841	25%	5051	4	5.1	5.7					14
18	F2841	25%	5245	4.5	5.1	7	8.2	10.7	12.5	14.4	42
18	F2841	25%	5246	4.3	5.9	7	8.3	10.8	13.2	15.5	42
19	CXB2971	25%	5286	6.1	8.1	9.6	11	13.3	15.5	18.1	42
19	CXB2971	25%	5287	5.3	6.1	7.3	8.3	10.1	11.4	13.4	42
2	F2995	35%	5223	3.9	4.7	5.9	7.1	8.9	10.1	12.4	42
2	F2995	35%	5224	5.2	6.5	7.5	8.3	9.7	11	14.1	42
5	CXB3429	35%	5204	2.5	2.4	2.5					14
5	CXB3429	35%	5205	3.1	4	5.5	6.8	8.7	10.7	12	42
5	CXB3429	35%	5206	2.5	3.4	4	3.6	4.3			28
7	CXB3064	35%	5194	4.5	5.9	7.8	7.8	10.1	12	12.8	42
7	CXB3064	35%	5195	4.6	6.1	8.1	10	12	13.8	15.5	42
7	CXB3064	35%	5196	4.2	5.8	6.8	8.8	10.9	12.5	14.7	42
12	P2143	35%	5229	6.9	8.5	10.5	12.9	15.1	16.5	18.1	42
12	P2143	35%	5230	6.5	7.7	9.5	13.6	14.5	16.8	19.8	42
15	CXB4513	35%	5062	4.2	6.2	7.9	8.8	8.8	9.8	11	42
15	CXB4513	35%	5063	3.7	4.7	4.9	5.7	7.1	7.8	8.4	42
15	CXB4513	35%	5064	4.2	6.2	7.2	8.7	9.8	10.7	12	42
17	CXB3202	35%	5132	3.8	5.6	7.1	9.3	11.6	14.4	16.9	42

17	CXB3202	35%	5133	3.4	4.8	5.7	5.9	6.8	8.2	8.9	42
17	CXB3202	35%	5134	4	6.1	7.4	9.1	11.4	14.2	16.4	42
21	CXB2112	35%	5120	3.8	5.7	7.2	8.8	11.2	14.5	17.5	42
21	CXB2112	35%	5172	5.1	7.3	8.5	10.1	11.4	12.8	15.7	42
21	CXB2112	35%	5173	6.5	7.5	9.4	10.5	11.9	13.1	14.8	42

APPENDIX 8

Blood Metabolite Data

EweID	Pen	Diet	Wk	NEFA meq/L	BHBA, mg/dL	PUN, mg/dL	D- lactate mg/dL	L- lactate, mg/dL		
W658	3	15% FNDF	0	707.05	10.58	17.44	65.94	139.06		
W658	3	15% FNDF	1	196.89	4.76	11.27	67.17	220.49		
W658	3	15% FNDF	2	107.93	6.44	9.51	62.05	212.54		
W658	3	15% FNDF	3	427.46	5.17	29.47	64.17	165.74		
W658	3	15% FNDF	4	94.32	3.57	10.74	67.00	162.56		
W658	3	15% FNDF	5	56.19	4.39	11.46	66.11	162.91		
W658	3	15% FNDF	6	62.54	12.63	14.79	67.70	1102.41		
CXB3690	4	15% FNDF	0	306.25	4.75	16.05	65.41	151.78		
CXB3690	4	15% FNDF	1	109.04	4.22	8.91	57.28	206.89		
CXB3690	4	15% FNDF	2	118.09	5.24	14.67	69.82	189.41		
CXB3690	4	15% FNDF	3	212.17	2.60	14.59	54.81	131.47		
CXB3690	4	15% FNDF	4	121.70	2.77	24.41	61.87	135.00		
CXB3690	4	15% FNDF	5	199.50	2.60	16.04	62.40	391.63		
CXB3690	4	15% FNDF	6	(This ewe was on the experiment for 5 wks.)						
CXB4238	8	15% FNDF	0	295.13	10.07	15.98	73.18	143.66		
CXB4238	8	15% FNDF	1	70.99	2.26	12.15	70.18	150.72		
CXB4238	8	15% FNDF	2	56.79	6.45	15.49	62.76	206.36		
CXB4238	8	15% FNDF	3	52.05	4.39	20.20	68.23	216.08		
CXB4238	8	15% FNDF	4	37.85	4.65	18.91	62.23	158.67		
CXB4238	8	15% FNDF	5	75.73	4.28	16.86	67.88	147.19		
CXB4238	8	15% FNDF	6	338.53	2.01	18.89	61.34	390.22		
F2808	9	15% FNDF	0	527.15	6.30	19.34	71.59	235.33		
F2808	9	15% FNDF	1	85.20	5.62	18.69	53.40	228.09		
F2808	9	15% FNDF	2	68.63	5.29	17.19	66.11	245.05		
F2808	9	15% FNDF	3	47.32	5.92	16.88	66.11	158.14		
F2808	9	15% FNDF	4	47.32	4.39	19.38	59.40	159.02		
F2808	9	15% FNDF	5	80.46	5.04	21.23	63.29	205.83		
F2808	9	15% FNDF	6	56.79	8.54	22.87	70.53	722.27		
CXB5045	11	15% FNDF	0	803.02	10.79	16.78	65.41	198.06		
CXB5045	11	15% FNDF	1	820.60	6.82	15.84	62.93	186.23		
CXB5045	11	15% FNDF	2	136.53	11.52	14.79	64.88	293.80		
CXB5045	11	15% FNDF	3	166.90	9.09	18.18	65.58	244.87		
CXB5045	11	15% FNDF	4	294.76	10.40	16.09	70.88	251.75		
CXB5045	11	15% FNDF	5	230.07	7.72	15.88	63.64	198.24		
CXB5045	11	15% FNDF	6	128.54	7.23	21.13	66.64	419.89		

CXB3432	13	15% FNDF	0	193.61	8.36	12.62	64.70	331.77
CXB3432	13	15% FNDF	1	119.11	5.32	20.98	62.58	196.29
CXB3432	13	15% FNDF	2	108.58	7.21	18.54	63.29	183.75
CXB3432	13	15% FNDF	3	39.75	5.69	13.16	64.70	246.64
CXB3432	13	15% FNDF	4	33.27	4.96	13.42	69.47	179.51
CXB3432	13	15% FNDF	5	60.80	8.12	17.89	65.23	174.21
CXB3432	13	15% FNDF	6	188.75	6.90	25.78	69.29	430.49
P2313	20	15% FNDF	0	376.83	7.69	14.49	63.29	167.33
P2313	20	15% FNDF	1	75.22	5.00	16.07	63.64	141.71
P2313	20	15% FNDF	2	85.25	6.58	16.52	65.41	156.90
P2313	20	15% FNDF	3	40.13	7.98	19.38	68.06	150.72
P2313	20	15% FNDF	4	17.58	6.56	11.68	66.11	142.24
P2313	20	15% FNDF	5	59.35	5.78	15.82	68.06	157.08
P2313	20	15% FNDF	6	269.89	6.17	15.27	68.23	374.68
Pu286	1	25% FNDF	0	184.18	7.45	16.68	67.17	157.79
Pu286	1	25% FNDF	1	73.44	4.10	11.50	62.76	139.95
Pu286	1	25% FNDF	2	78.88	8.86	12.71	63.64	282.85
Pu286	1	25% FNDF	3	55.28	5.79	14.77	65.05	182.69
Pu286	1	25% FNDF	4	75.25	5.29	16.21	65.05	133.94
Pu286	1	25% FNDF	5	87.96	4.25	16.15	59.05	133.24
Pu286	1	25% FNDF	6	34.40	8.78	21.37	66.64	626.20
CXB3496	6	25% FNDF	0	232.07	6.01	19.77	65.94	162.03
CXB3496	6	25% FNDF	1	89.14	6.40	15.82	65.05	296.45
CXB3496	6	25% FNDF	2	62.00	4.22	14.88	70.88	199.12
CXB3496	6	25% FNDF	3	53.86	10.91	15.82	66.29	239.22
CXB3496	6	25% FNDF	4	78.28	4.69	19.59	63.82	150.55
CXB3496	6	25% FNDF	5	74.66	7.66	18.98	69.12	140.30
CXB3496	6	25% FNDF	6	83.71	12.19	16.82	68.94	821.21
P2309	10	25% FNDF	0	403.45	6.67	14.84	68.06	333.00
P2309	10	25% FNDF	1	358.69	6.45	22.83	69.12	162.73
P2309	10	25% FNDF	2	270.79	3.38	24.98	48.98	138.35
P2309	10	25% FNDF	3	51.82	6.01	22.70	65.94	133.59
P2309	10	25% FNDF	4	45.43	5.94	14.49	67.53	137.83
P2309	10	25% FNDF	5	37.44	5.72	21.33	65.23	156.55
P2309	10	25% FNDF	6	90.18	2.80	20.78	60.46	347.82
F3011	14	25% FNDF	0	37.15	8.78	9.06	66.29	332.30
F3011	14	25% FNDF	1	148.26	2.83	15.20	63.64	194.17
F3011	14	25% FNDF	2	399.30	5.73	35.37	65.76	282.67
F3011	14	25% FNDF	3	72.14	6.66	13.73	69.12	243.10
F3011	14	25% FNDF	4	117.49	4.90	20.02	65.94	160.08
F3011	14	25% FNDF	5	64.04	5.08	18.83	65.23	169.27
F3011	14	25% FNDF	6	89.15	3.02	18.93	65.41	371.85
CXB2979	16	25% FNDF	0	313.69	8.78	13.09	65.41	271.89

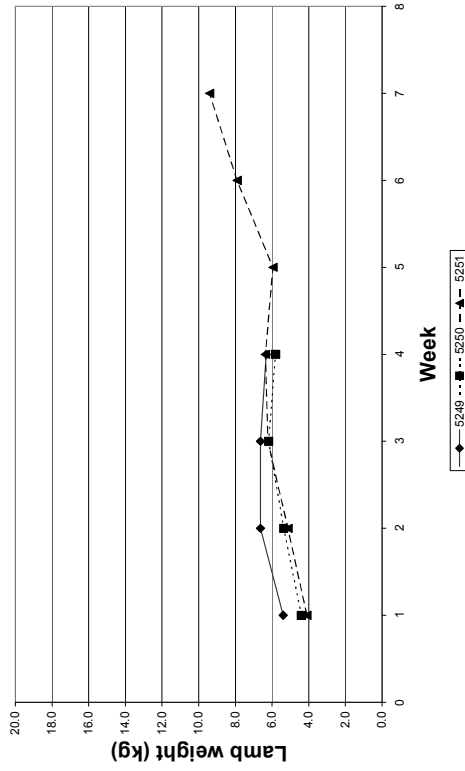
CXB2979	16	25% FNDF	1	150.42	2.83	18.65	65.94	156.55
CXB2979	16	25% FNDF	2	156.11	5.73	23.55	64.70	207.78
CXB2979	16	25% FNDF	3	64.99	6.66	25.06	67.35	140.48
CXB2979	16	25% FNDF	4	63.09	4.90	18.01	63.29	155.84
CXB2979	16	25% FNDF	5	47.90	5.08	22.79	65.05	151.43
CXB2979	16	25% FNDF	6	93.46	3.02	23.87	64.17	342.88
F2841	18	25% FNDF	0	621.24	9.62	26.64	64.70	132.35
F2841	18	25% FNDF	1	144.72	9.77	20.33	64.52	262.89
F2841	18	25% FNDF	2	53.60	7.07	21.78	64.88	224.91
F2841	18	25% FNDF	3	25.12	6.74	24.06	63.11	180.04
F2841	18	25% FNDF	4	42.21	5.08	25.74	69.82	159.55
F2841	18	25% FNDF	5	121.94	6.93	19.57	63.46	172.80
F2841	18	25% FNDF	6	152.32	5.06	23.59	63.82	440.38
CXB2791	19	25% FNDF	0	144.57	7.63	11.86	64.70	156.90
CXB2791	19	25% FNDF	1	104.47	7.22	16.60	58.34	160.44
CXB2791	19	25% FNDF	2	95.28	9.27	20.90	67.53	158.85
CXB2791	19	25% FNDF	3	37.63	9.21	17.95	65.05	179.16
CXB2791	19	25% FNDF	4	40.13	9.56	18.13	63.82	165.91
CXB2791	19	25% FNDF	5	127.86	8.22	19.92	64.52	139.06
CXB2791	19	25% FNDF	6	67.71	6.01	18.69	62.58	388.10
F2995	2	35% FNDF	0	501.90	7.02	25.21	69.82	237.63
F2995	2	35% FNDF	1	129.72	5.79	19.57	67.35	257.41
F2995	2	35% FNDF	2	109.75	6.34	16.66	63.29	227.21
F2995	2	35% FNDF	3	77.07	6.46	18.52		189.94
F2995	2	35% FNDF	4	50.74	9.17	20.84	59.40	127.58
F2995	2	35% FNDF	5	93.41	5.83	20.23	61.34	167.15
F2995	2	35% FNDF	6	61.64	8.16	29.43	60.29	777.40
CXB3429	5	35% FNDF	0	413.00	6.20	18.03	65.94	195.94
CXB3429	5	35% FNDF	1	261.02	1.45	16.80	63.82	143.13
CXB3429	5	35% FNDF	2	65.62	8.49	18.71		226.68
CXB3429	5	35% FNDF	3	60.19	7.35	17.38	62.58	200.53
CXB3429	5	35% FNDF	4	70.14	4.84	23.03	59.58	132.88
CXB3429	5	35% FNDF	5	83.71	5.63	16.29	60.11	146.31
CXB3429	5	35% FNDF	6	215.79	8.38	52.77	65.23	694.74
CXB3064	7	35% FNDF	0	263.56	4.20	15.39	63.82	135.71
CXB3064	7	35% FNDF	1	225.68	8.11	13.85	67.53	255.29
CXB3064	7	35% FNDF	2	78.10	8.15	17.50	67.17	147.19
CXB3064	7	35% FNDF	3	64.68	10.00	20.76	69.82	188.88
CXB3064	7	35% FNDF	4	85.20	4.76	21.17	65.23	128.29
CXB3064	7	35% FNDF	5	176.75	2.97	24.67	64.17	136.94
CXB3064	7	35% FNDF	6	274.61	5.14	19.98	72.30	539.28
P2143	12	35% FNDF	0	897.32	6.06	15.43	67.35	123.87
P2143	12	35% FNDF	1	158.91	5.06	13.50	60.11	157.61

P2143	12	35% FNDF	2	75.00	6.45	17.75	63.46	162.56
P2143	12	35% FNDF	3	70.20	8.36	19.98	67.53	146.13
P2143	12	35% FNDF	4	61.41	8.36	20.35	63.99	186.23
P2143	12	35% FNDF	5	67.80	6.75	23.67	63.82	127.23
P2143	12	35% FNDF	6	80.59	5.19	22.07	67.35	363.37
CXB4513	15	35% FNDF	0	1037.44	14.43	15.51	67.88	266.42
CXB4513	15	35% FNDF	1	306.99	9.82	16.56	66.82	184.99
CXB4513	15	35% FNDF	2	378.25	5.99	22.71	64.35	188.70
CXB4513	15	35% FNDF	3	73.76	6.36	23.98	68.59	245.93
CXB4513	15	35% FNDF	4	159.60	7.14	16.19	62.23	216.43
CXB4513	15	35% FNDF	5	115.87	10.18	26.64	66.11	294.50
CXB4513	15	35% FNDF	6	113.44	7.79	22.66	67.17	522.34
CXB3202	17	35% FNDF	0	446.58	6.26	12.87	71.77	181.63
CXB3202	17	35% FNDF	1	313.69	9.34	16.70	63.46	294.67
CXB3202	17	35% FNDF	2	150.42	7.49	24.16	66.47	168.91
CXB3202	17	35% FNDF	3	101.06	6.48	26.76	64.17	140.65
CXB3202	17	35% FNDF	4	76.38	5.47	19.84	68.76	145.95
CXB3202	17	35% FNDF	5	39.36	10.05	25.68	64.70	300.69
CXB3202	17	35% FNDF	6	73.53	5.30	23.18	65.94	348.53
CXB2112	21	35% FNDF	0	335.05	6.83	12.71	66.47	122.81
CXB2112	21	35% FNDF	1	122.85	8.55	20.37	63.11	149.13
CXB2112	21	35% FNDF	2	57.68	9.09	20.57	67.00	169.80
CXB2112	21	35% FNDF	3	47.65	11.32	16.19	67.53	206.89
CXB2112	21	35% FNDF	4	208.06	7.34	24.80	59.05	150.90
CXB2112	21	35% FNDF	5	120.34	5.82	23.03	62.23	148.96
CXB2112	21	35% FNDF	6	57.68	7.96	21.45	63.11	354.19

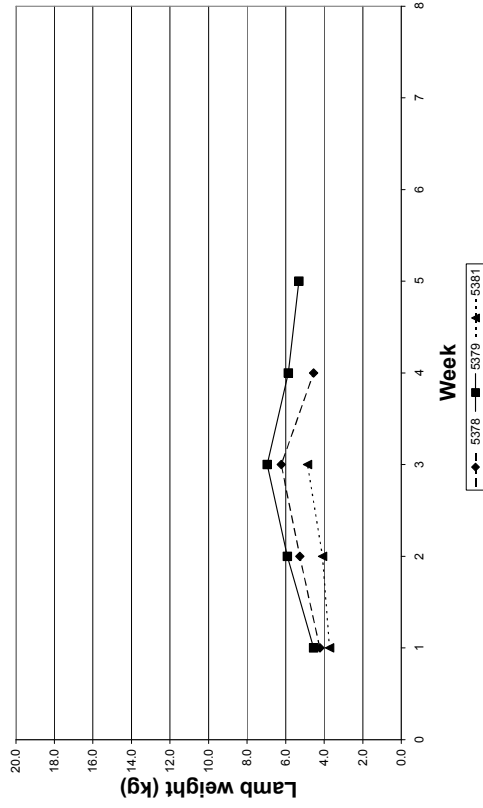
APPENDIX 9

Lamb Weights By Pen

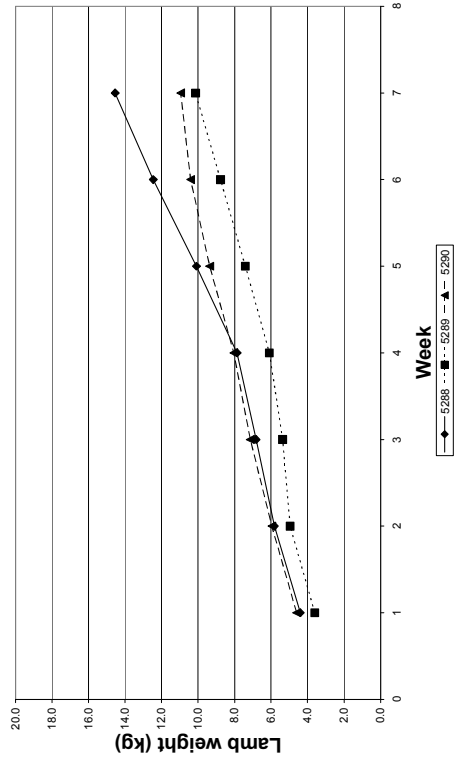
Lamb Weights for Ewe 3 - 15% FNDF



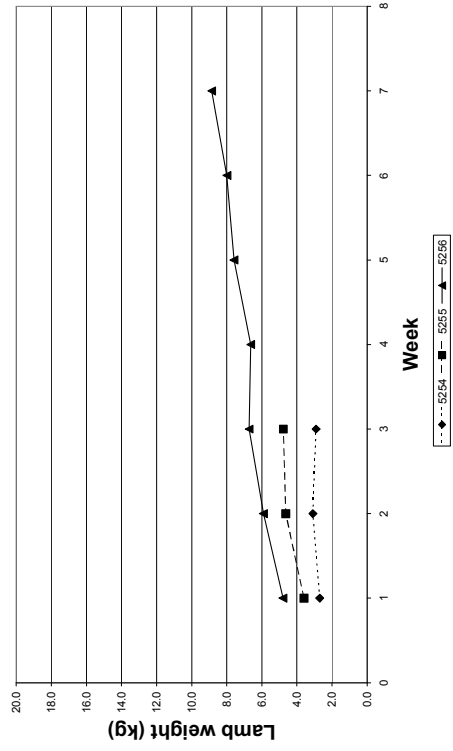
Lamb Weights for Ewe 4 - 15% FNDF



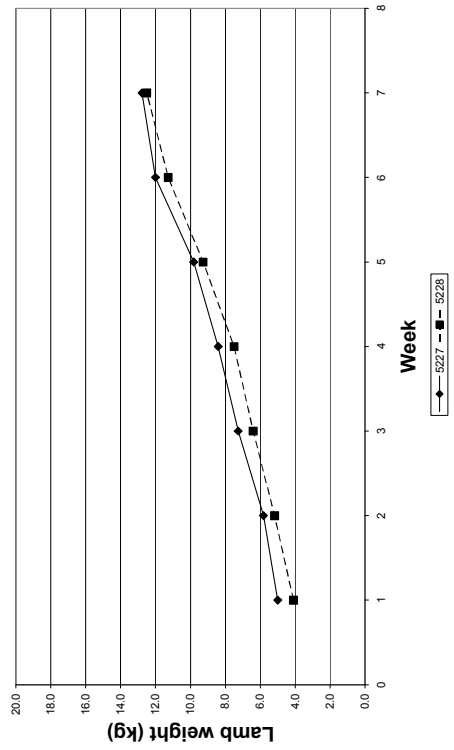
Lamb Weights for Ewe 9 - 15% FNDF



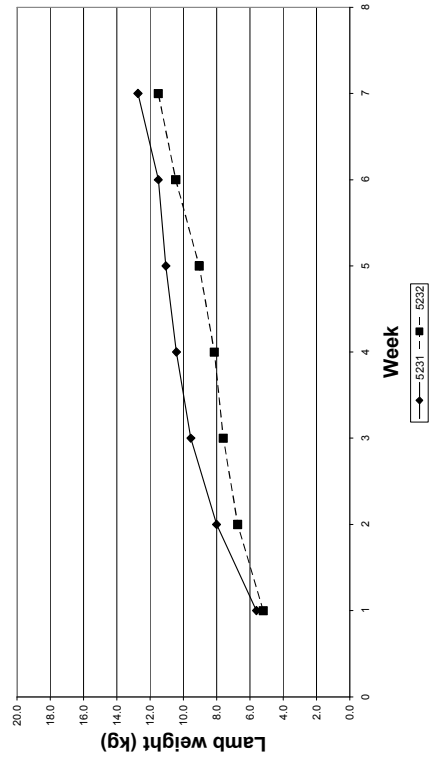
Lamb Weights for Ewe 13 - 15% FNDF



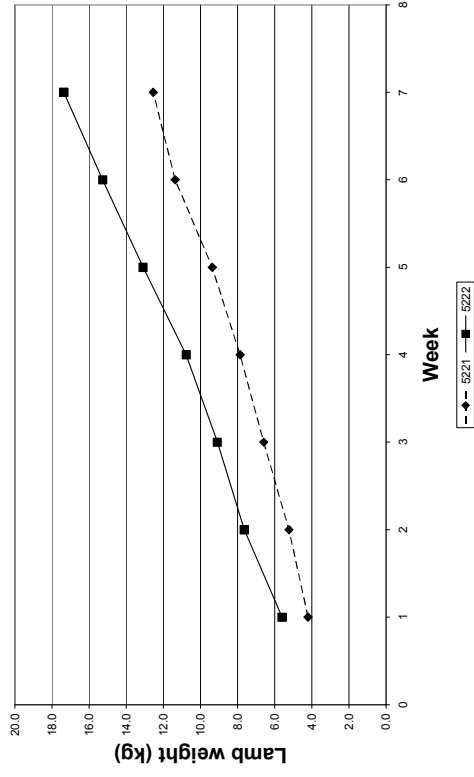
Lamb Weights for Ewe 8 - 15% FNDF



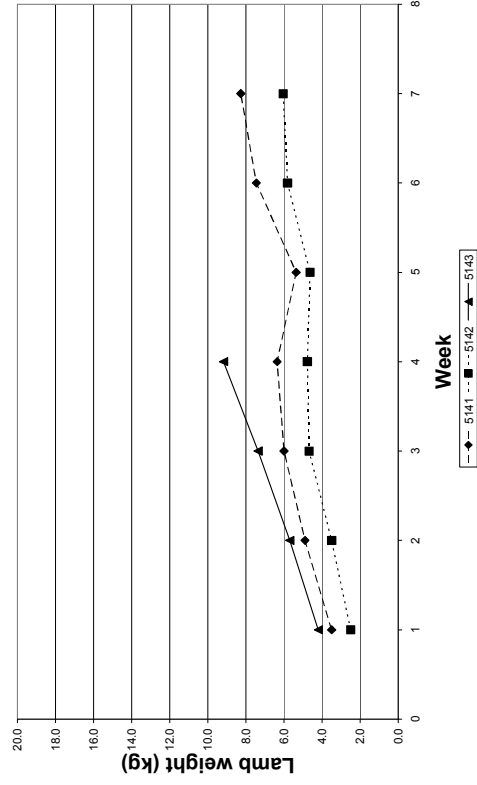
Lamb Weights for Ewe 11 - 15% FNDF



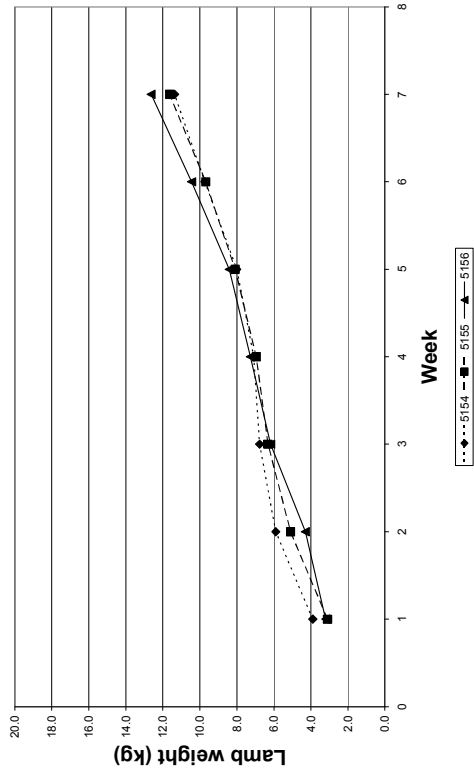
Lamb Weights for Ewe 1 - 25% FNDF



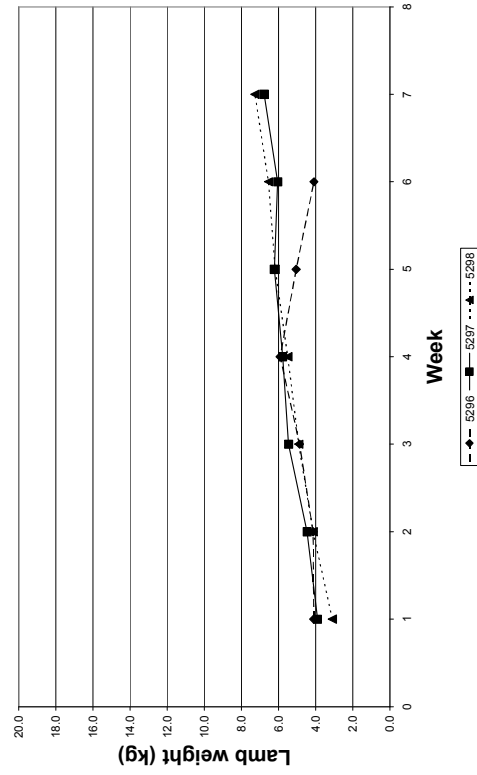
Lamb Weights for Ewe 10 - 25% FNDF



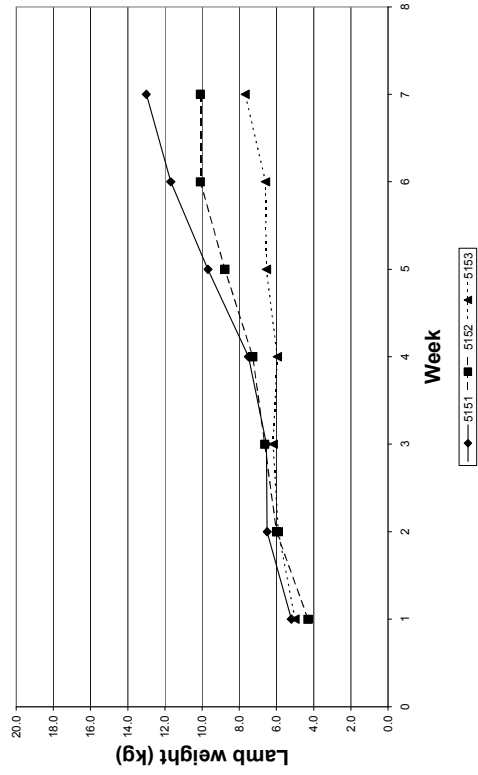
Lamb Weights for Ewe 20 - 15% FNDF



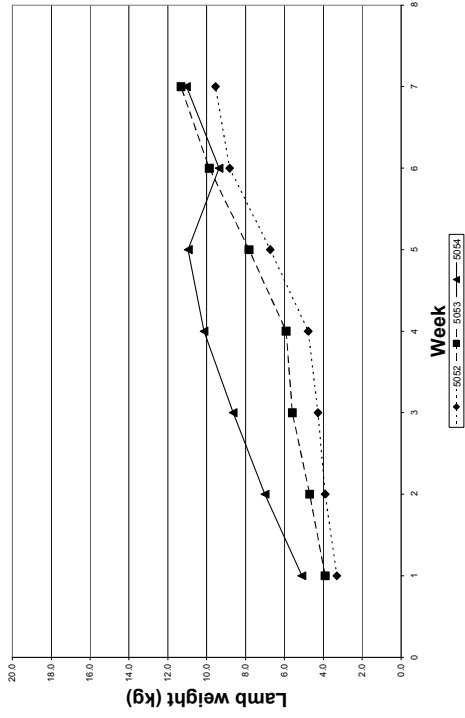
Lamb Weights for Ewe 6 - 25% FNDF



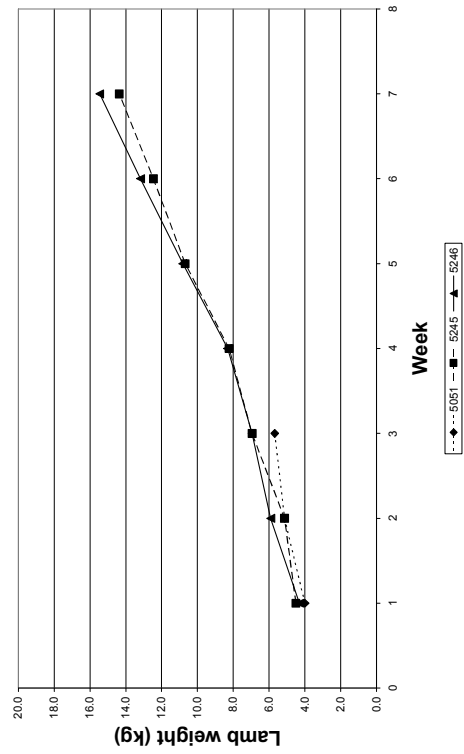
Lamb Weights for Ewe 14 - 25% FNDF



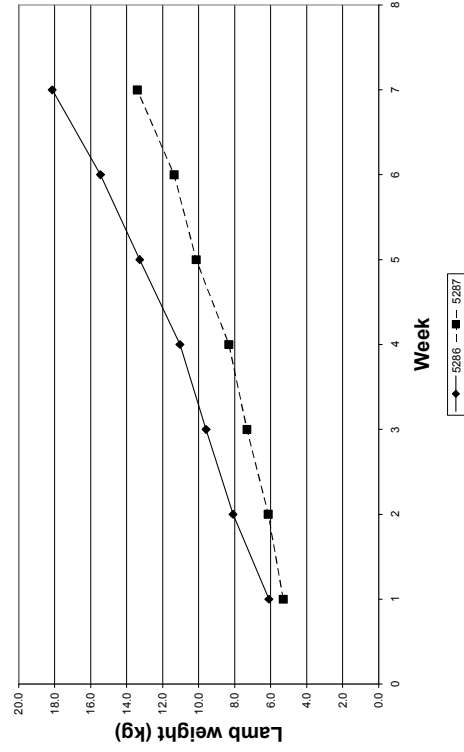
Lamb Weights for Ewe 16 - 25% FNDF



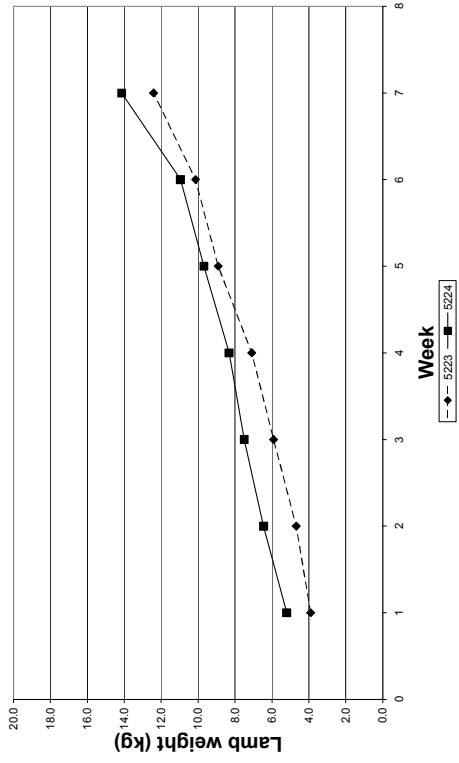
Lamb Weights for Ewe 18 - 25% FNDF



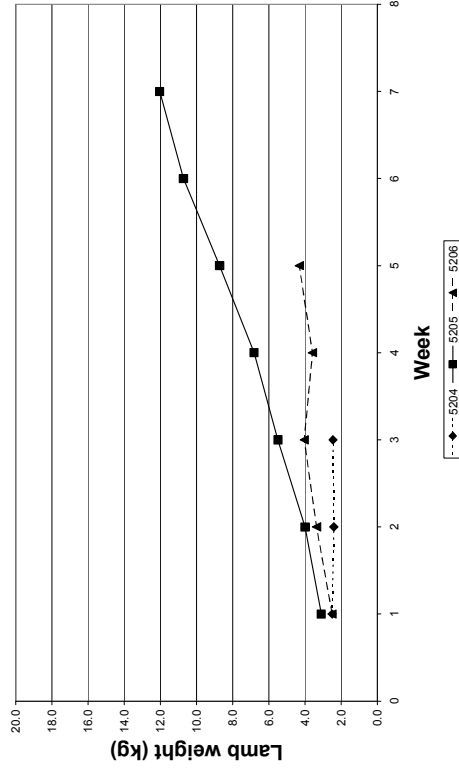
Lamb Weights for Ewe 19 - 25% FNDF



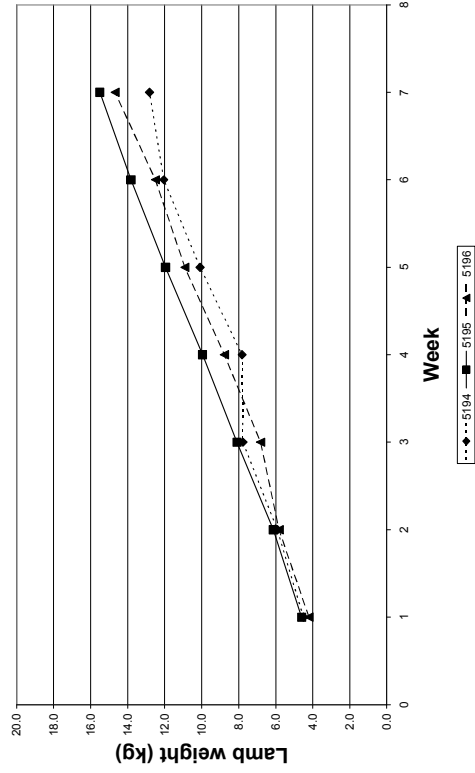
Lamb Rates of Gain for Ewe 2 - 35% FNDF



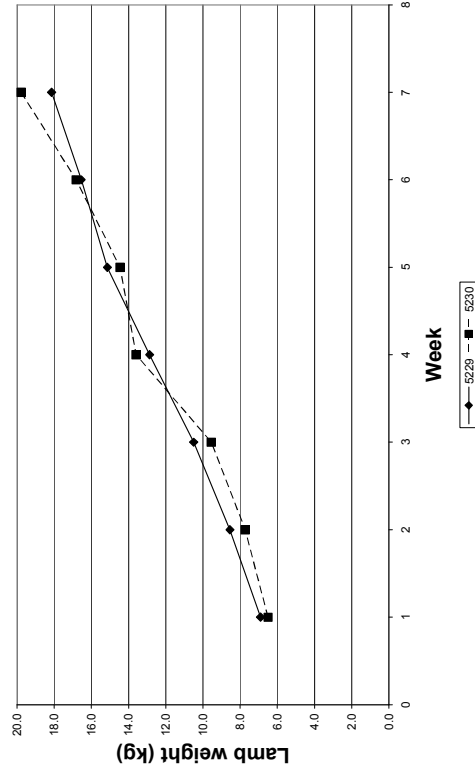
Lamb Weights for Ewe 5 - 35% FNDF



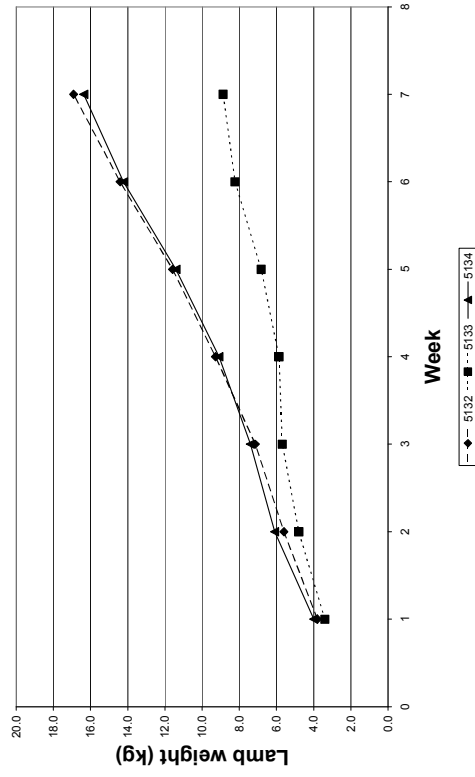
Lamb Weights for Ewe 7 - 35% FNDF



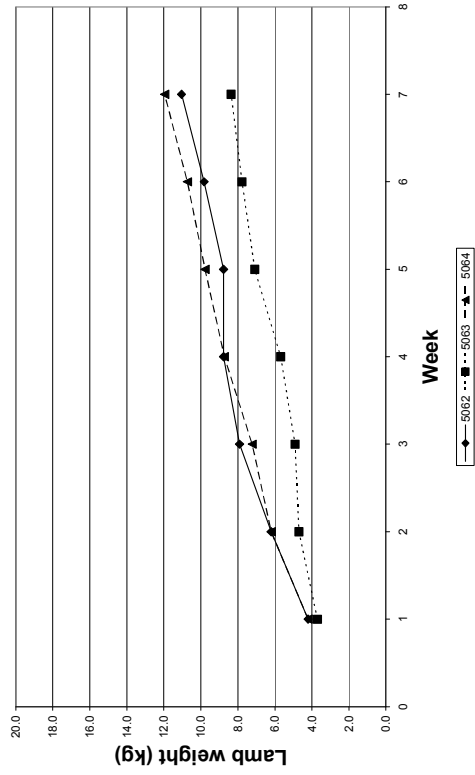
Lamb Weights for Ewe 12 - 35% FNDF



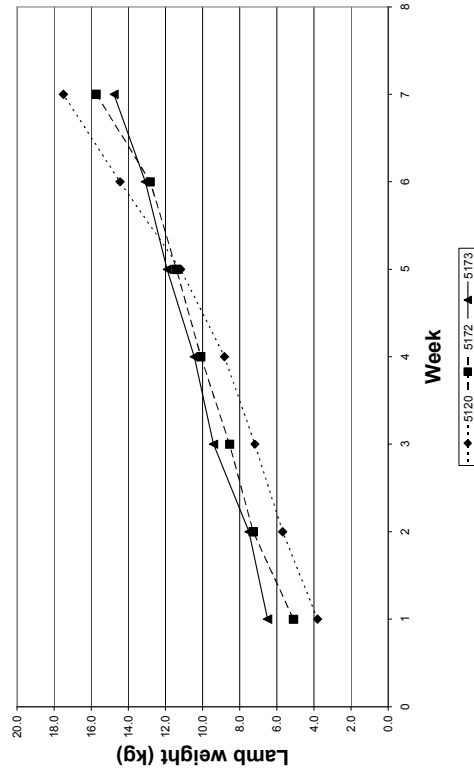
Lamb Weights for Ewe 17 - 35% FNDF



Lamb Weights for Ewe 15 - 35% FNDF

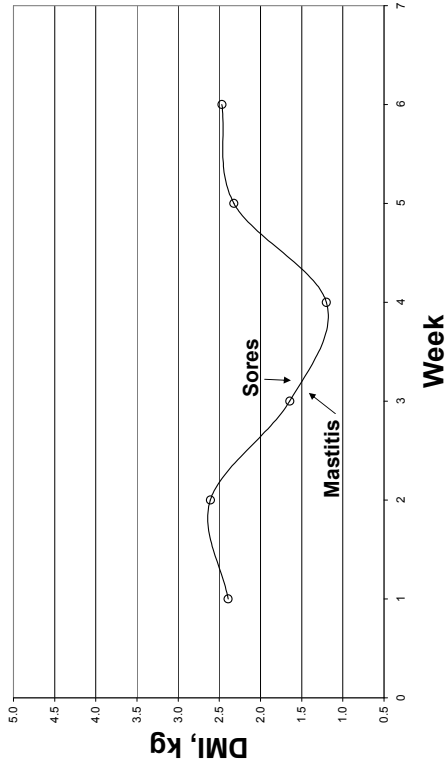
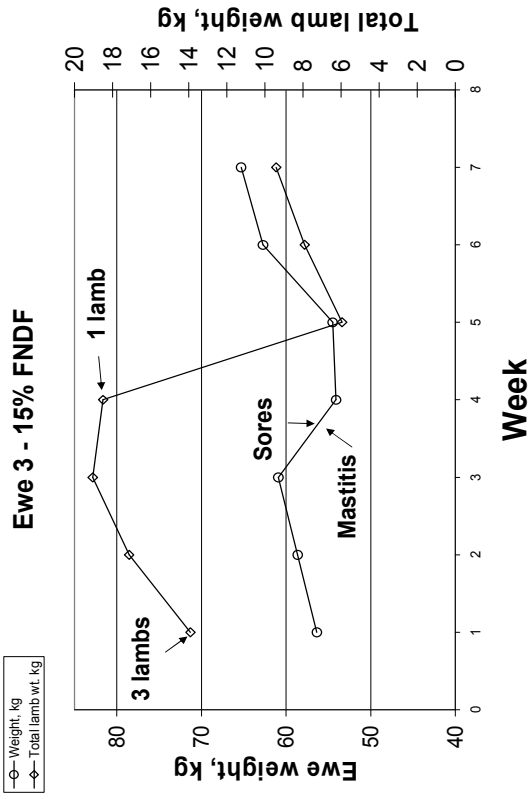


Lamb Weights for Ewe 21 - 35% FNDF

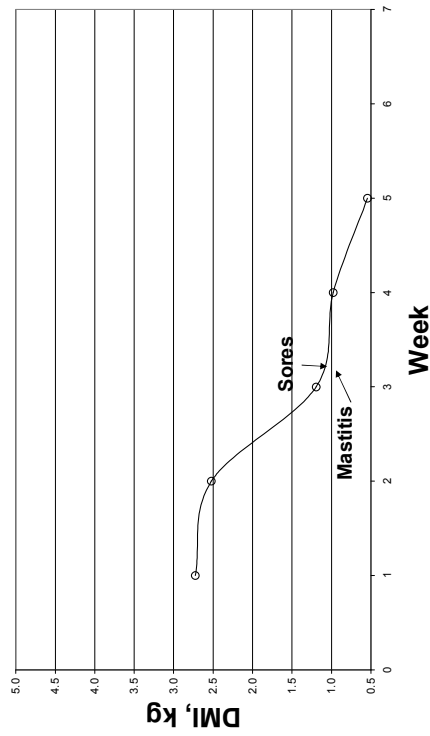


APPENDIX 10

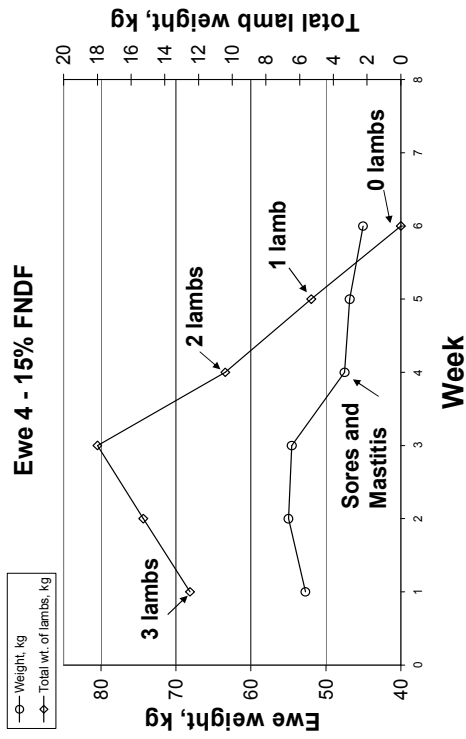
Ewe charts for weekly DMI, body weights and kilograms of lambs throughout the experiment, with indicators for start of mastitis and appearance of sores.



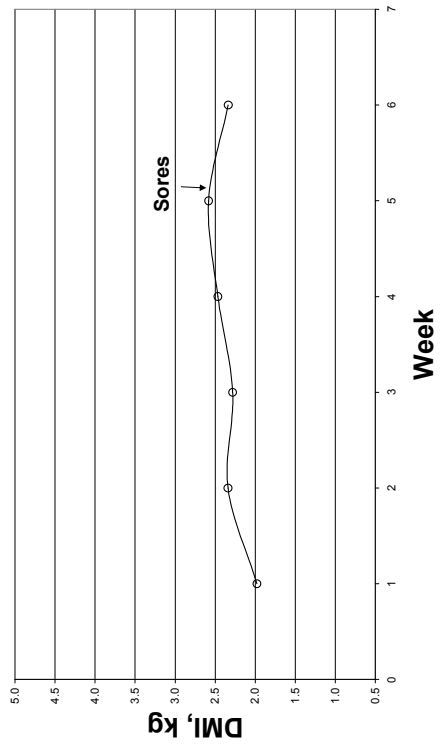
Ewe 4 - 15% FNDF



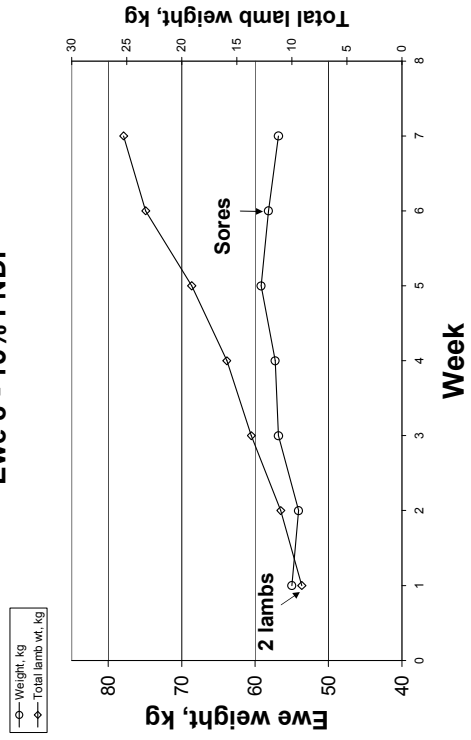
Ewe 4 - 15% FNDF



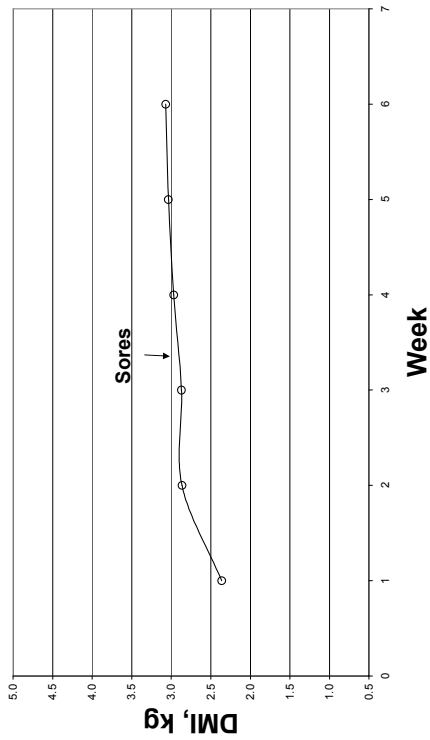
Ewe 8 - 15% FNDF



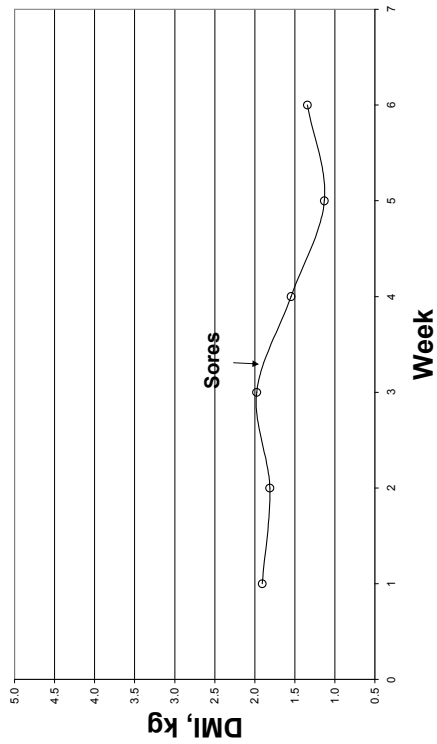
Ewe 8 - 15% FNDF



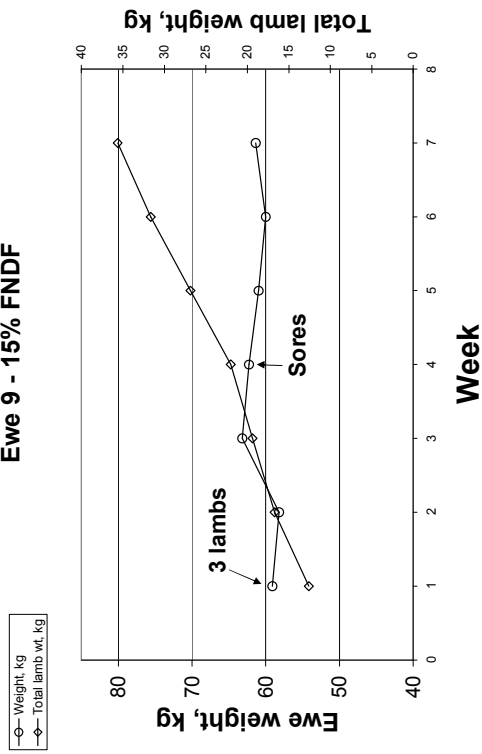
Ewe 9 - 15% FNDF



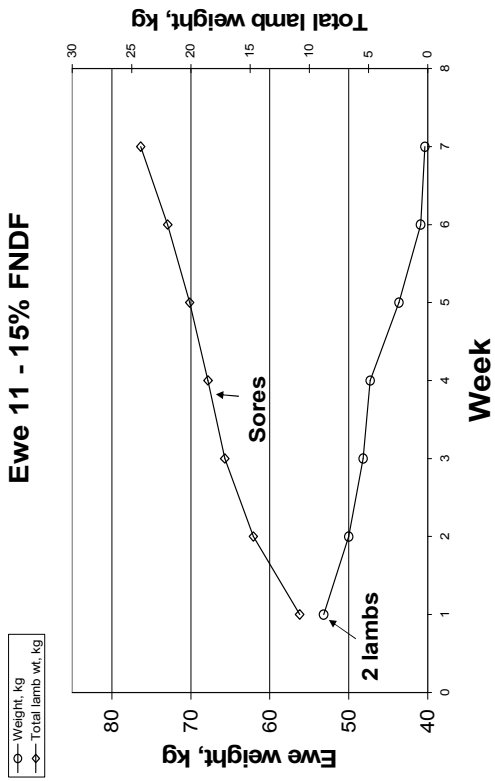
Ewe 11 - 15% FNDF



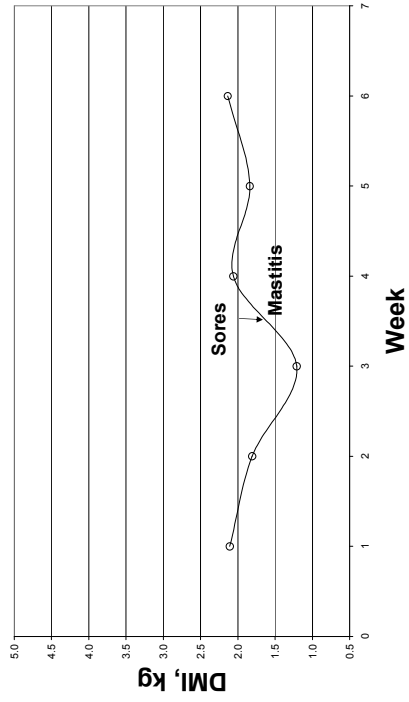
Ewe 9 - 15% FNDF



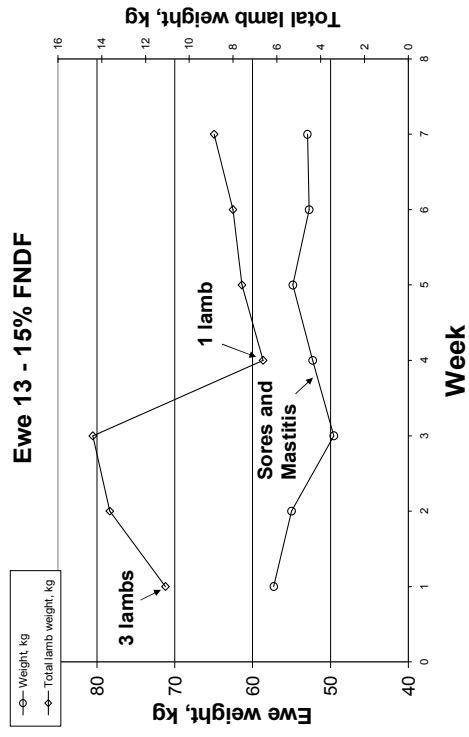
Ewe 11 - 15% FNDF



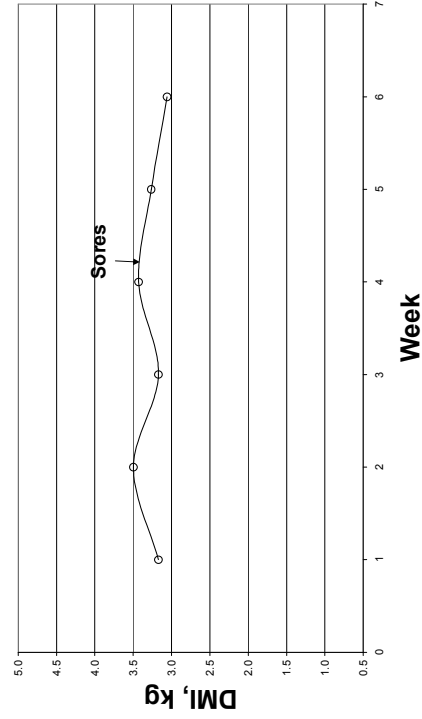
Ewe 13 - 15% FNDF



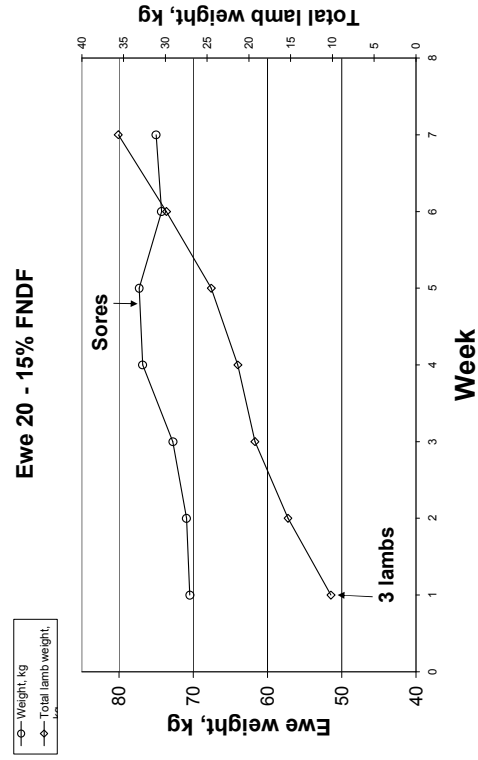
Ewe 13 - 15% FNDF



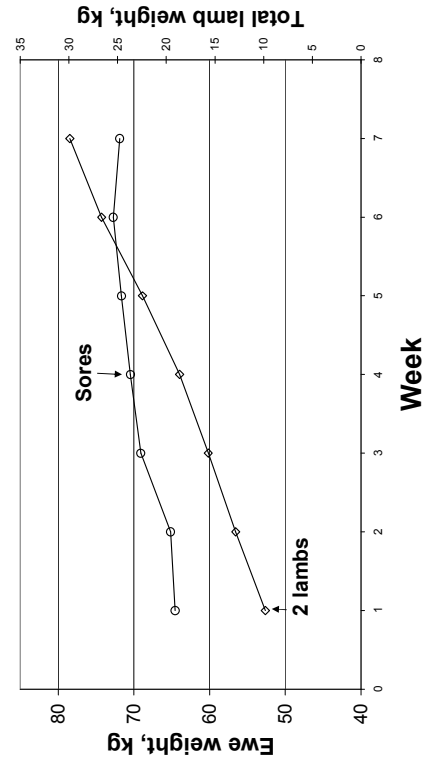
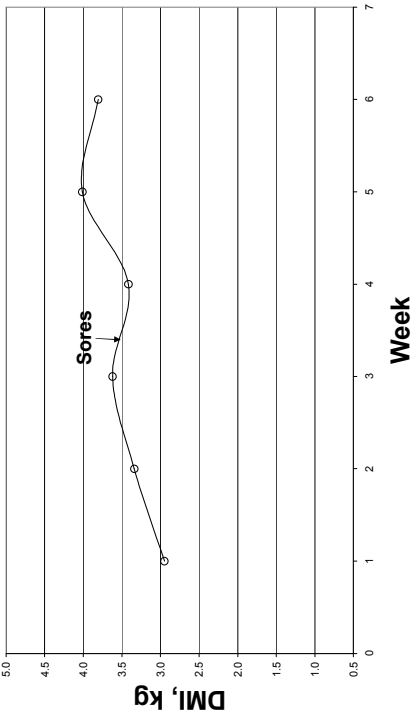
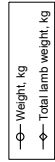
Ewe 20 - 15% FNDF



Ewe 20 - 15% FNDF

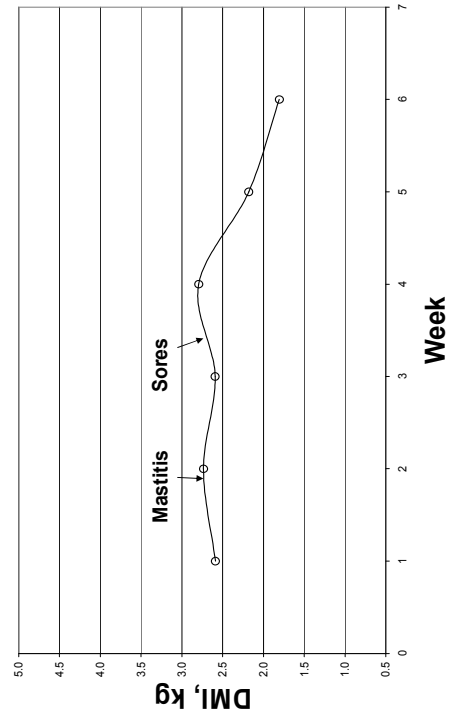


Ewe 1 - 25% FNDF

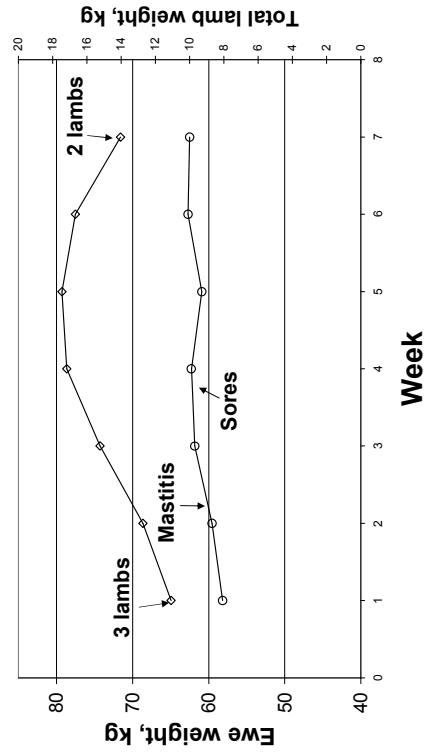
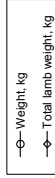


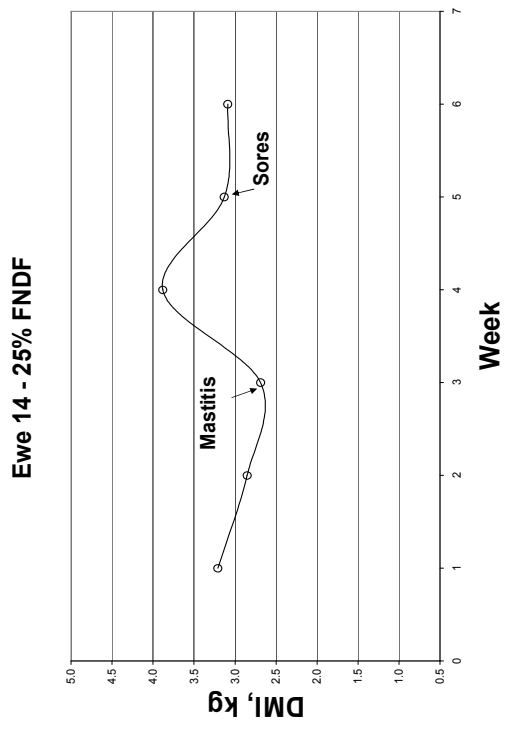
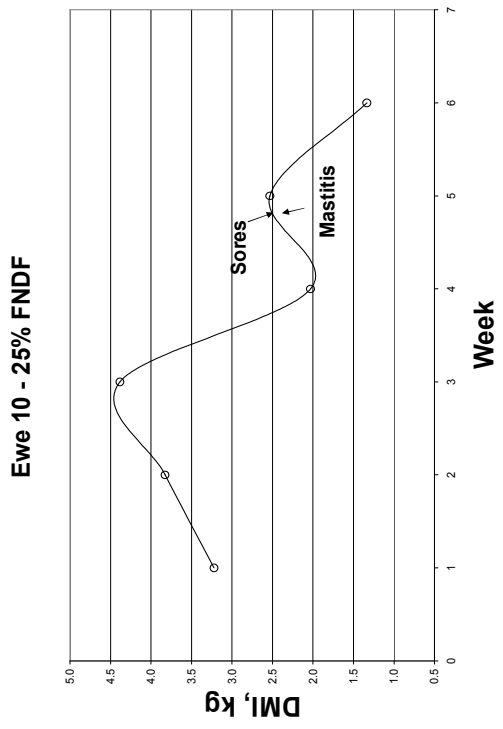
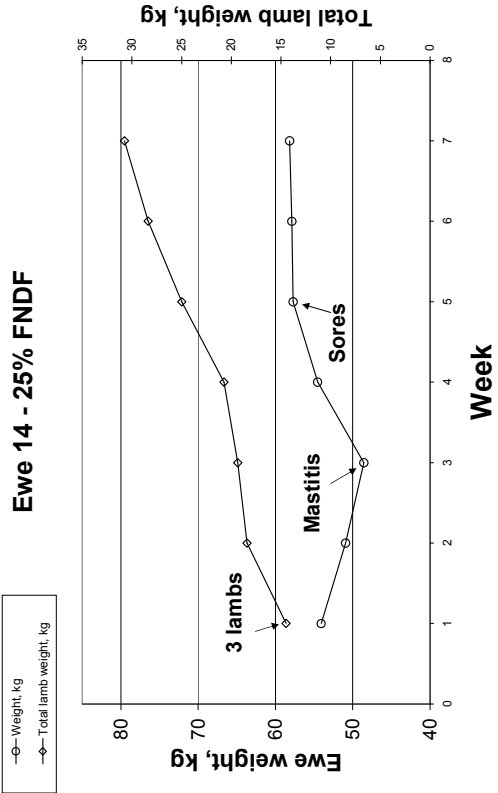
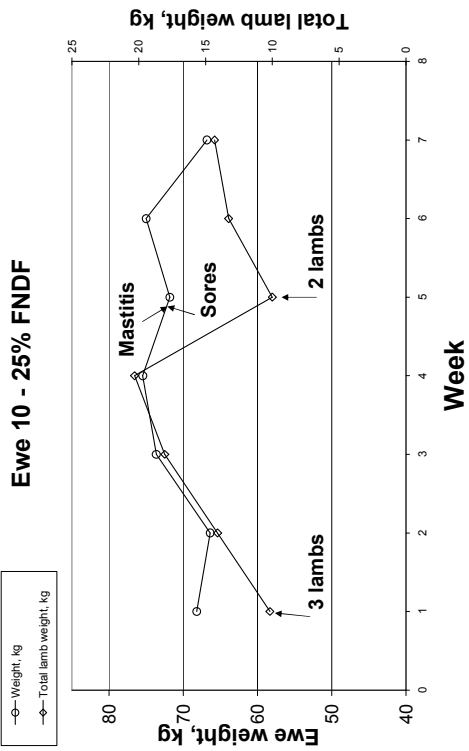
Ewe 1 - 25% FNDF

Ewe 6 - 25% FNDF

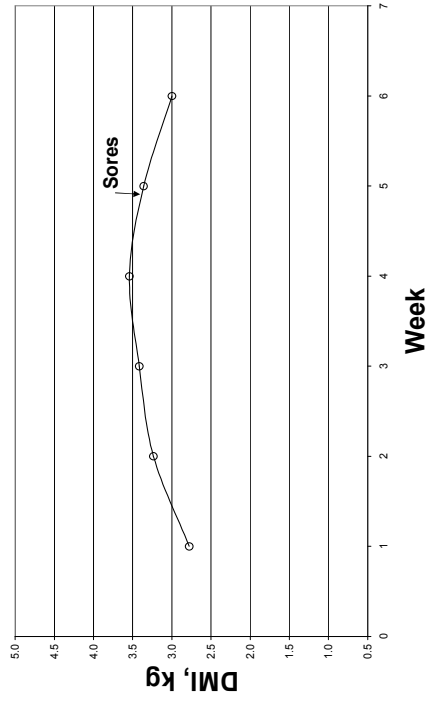


Ewe 6 - 25% FNDF

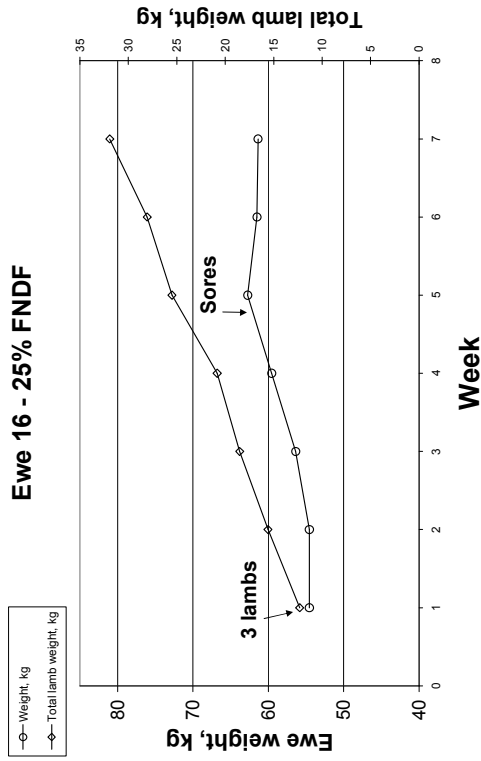




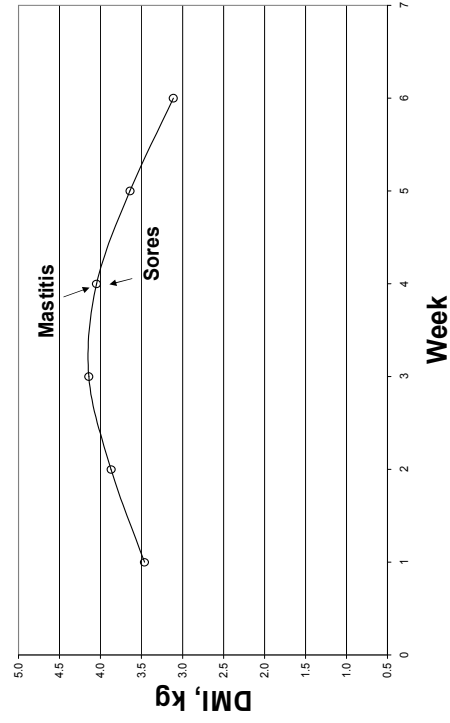
Ewe 16 - 25% FNDF



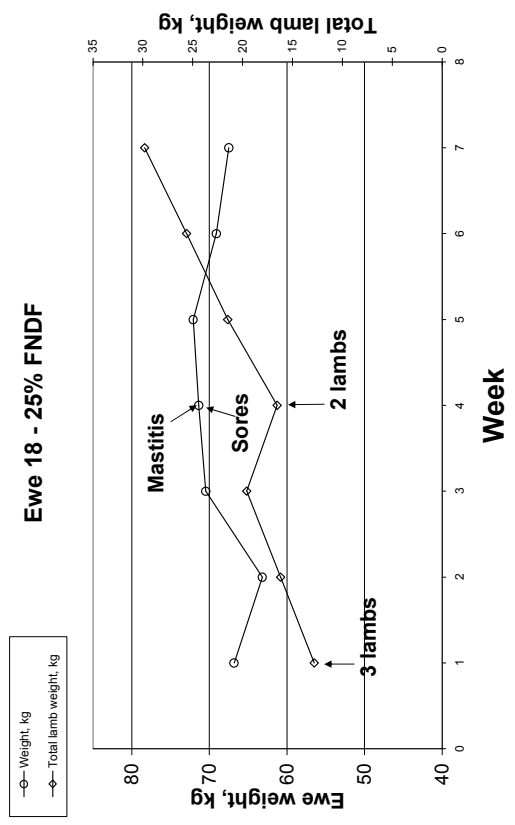
Ewe 16 - 25% FNDF



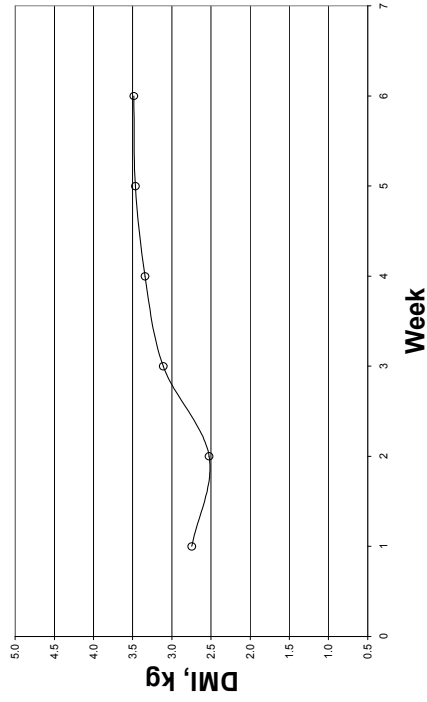
Ewe 18 - 25% FNDF



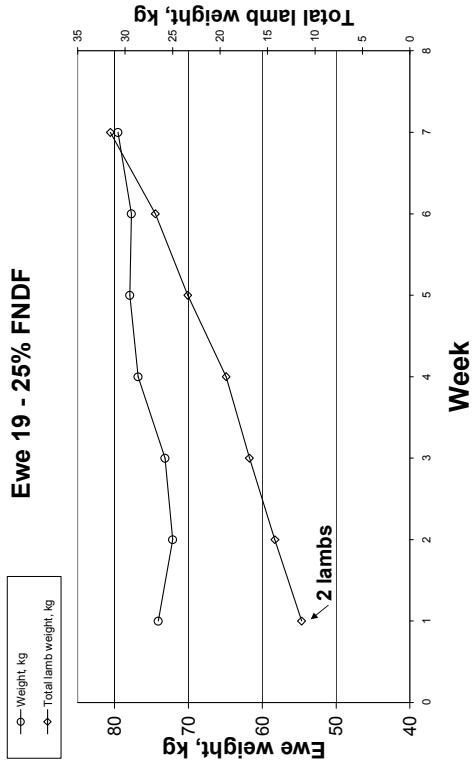
Ewe 18 - 25% FNDF



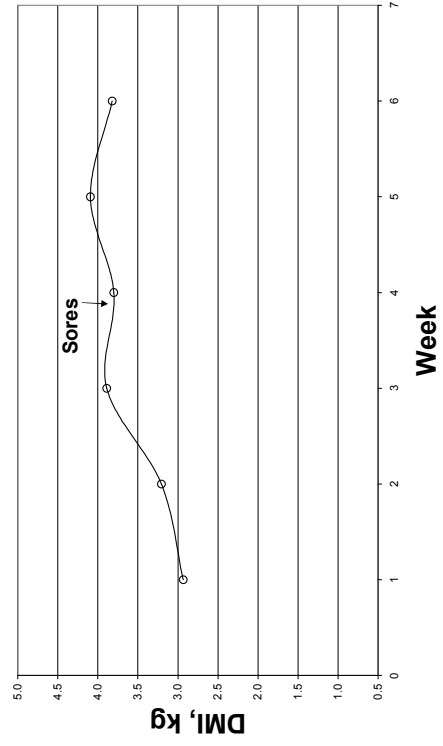
Ewe 19 - 25% FNDF



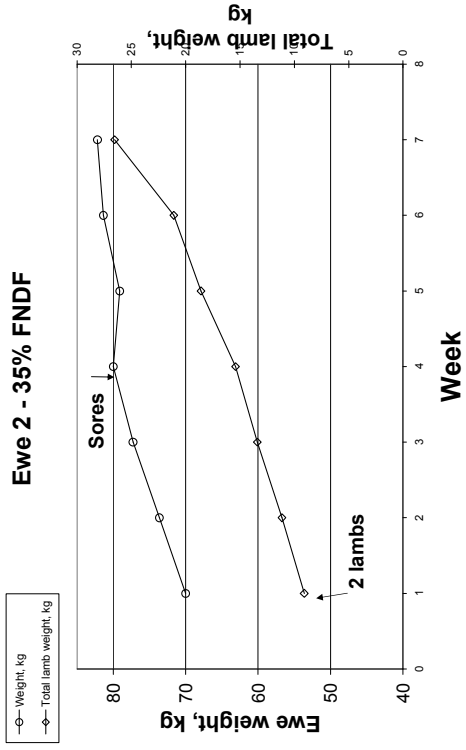
Ewe 19 - 25% FNDF

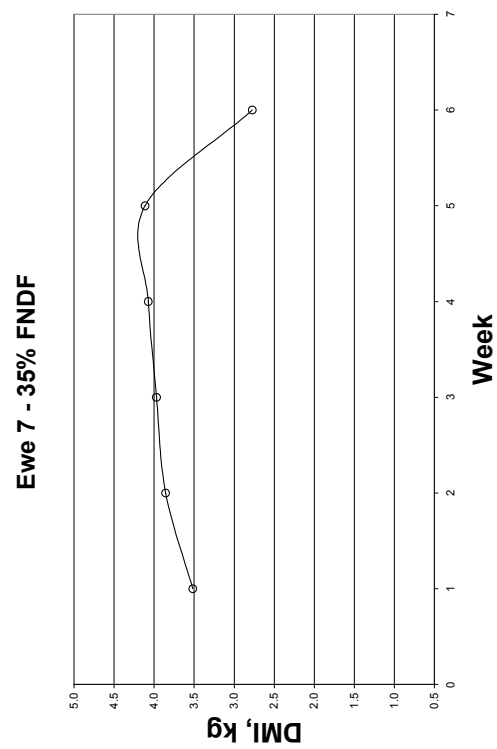
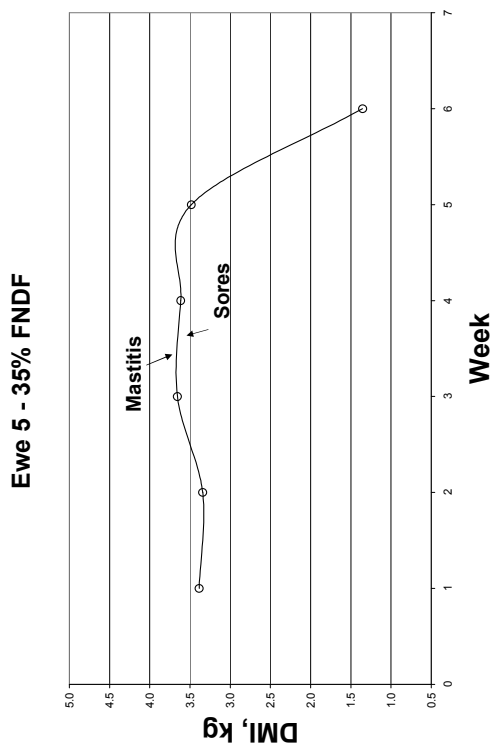
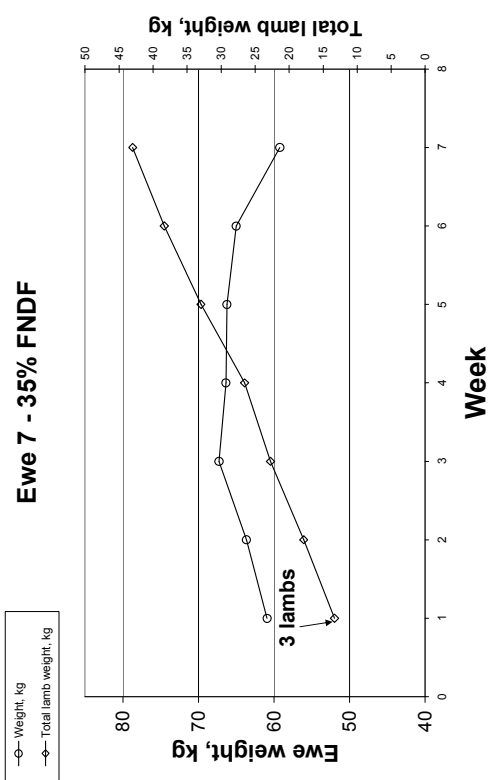
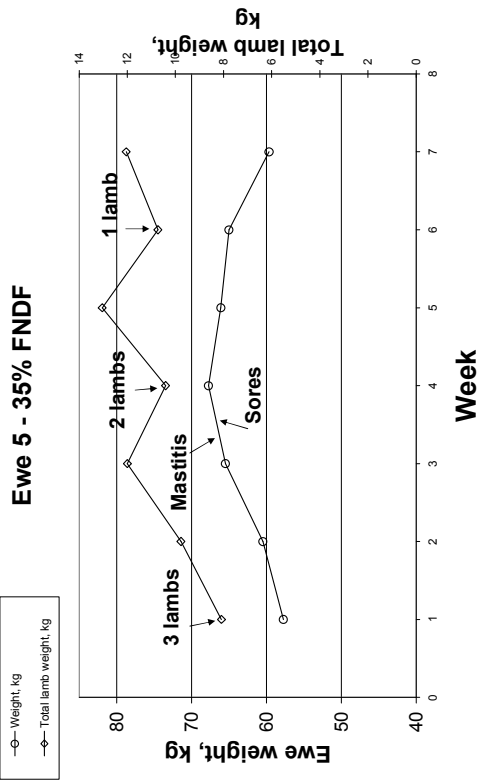


Ewe 2 - 35% FNDF

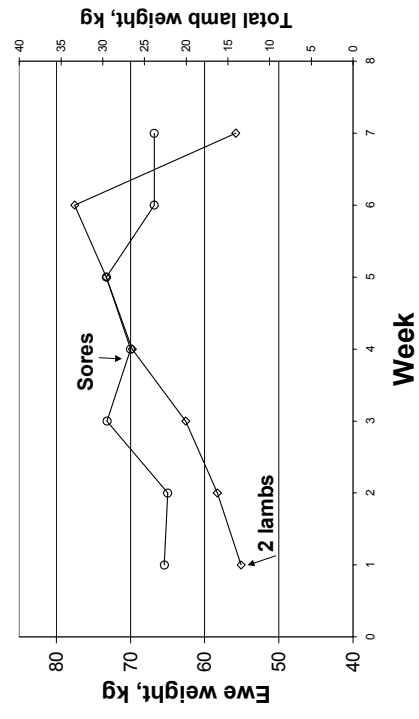
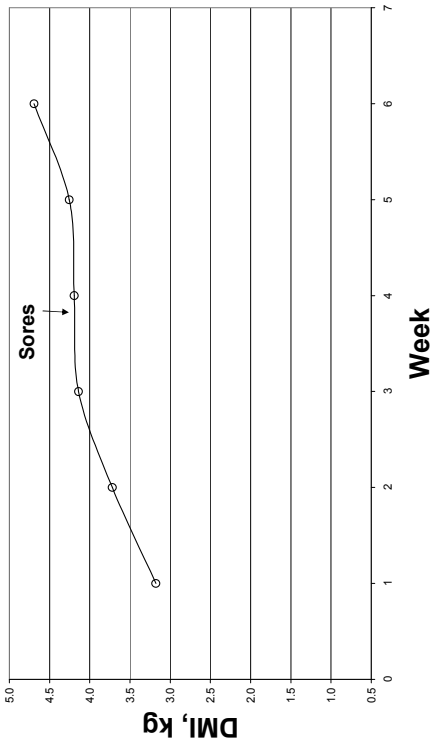
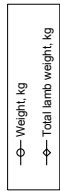


Ewe 2 - 35% FNDF

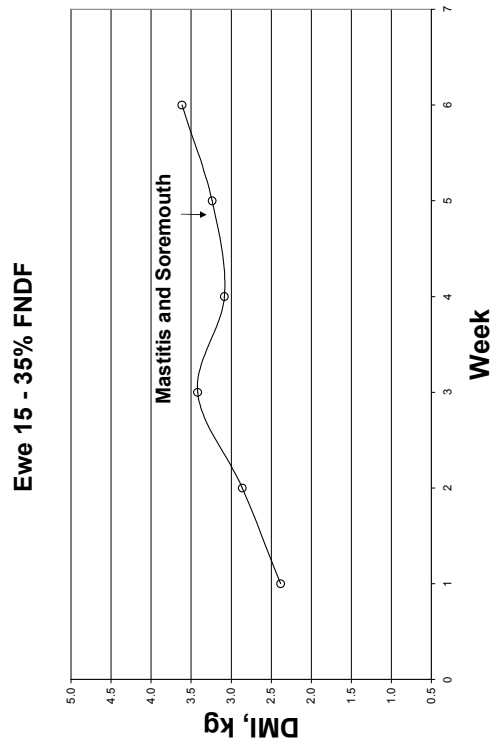




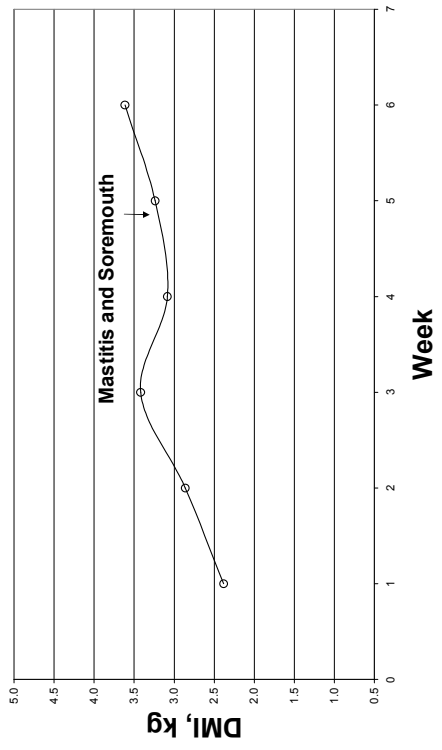
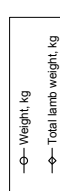
Ewe 12 - 35% FNDF



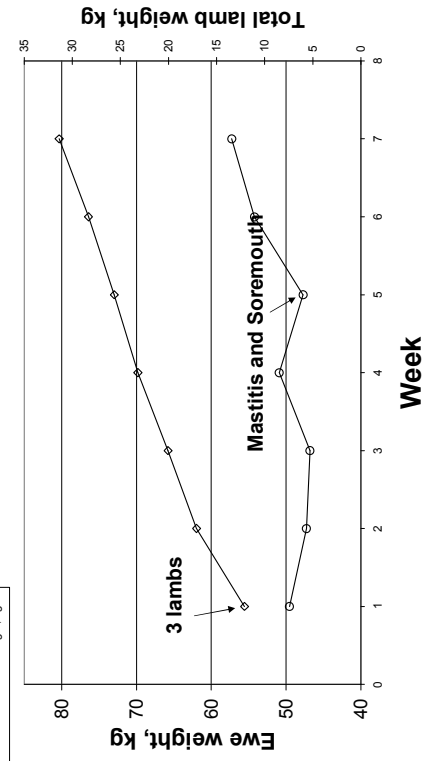
Ewe 12 - 35% FNDF



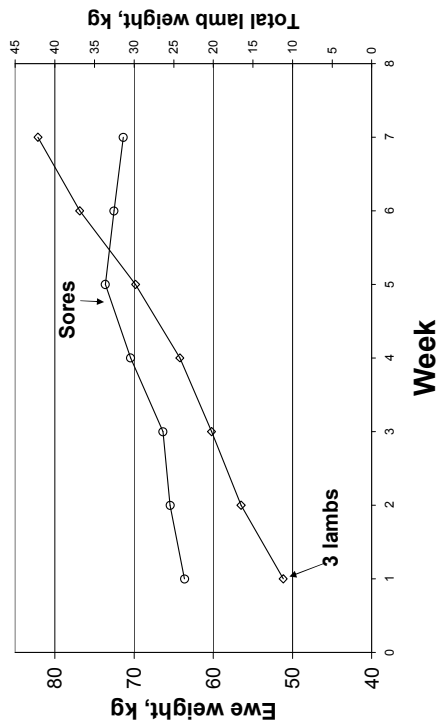
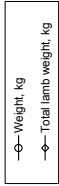
Ewe 15 - 35% FNDF



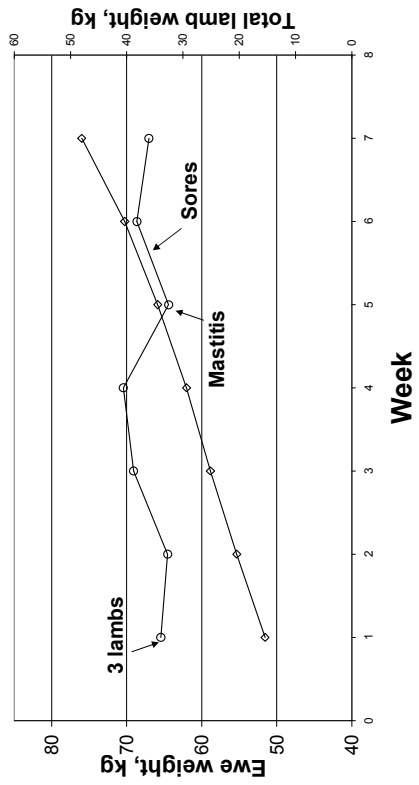
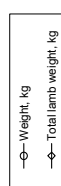
Ewe 15 - 35% FNDF



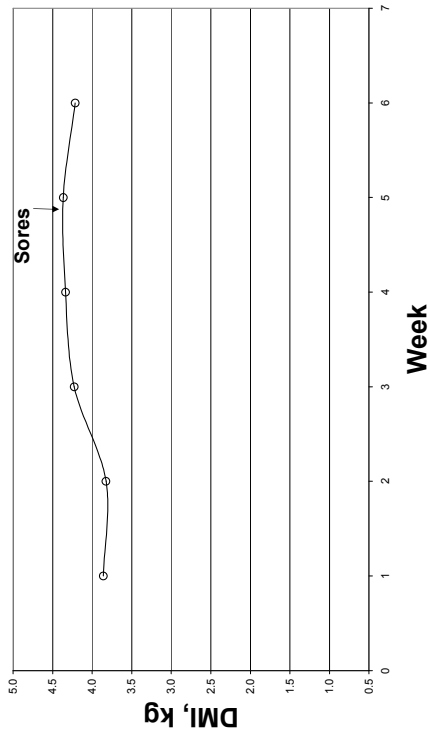
Ewe 17 - 35% FNDF



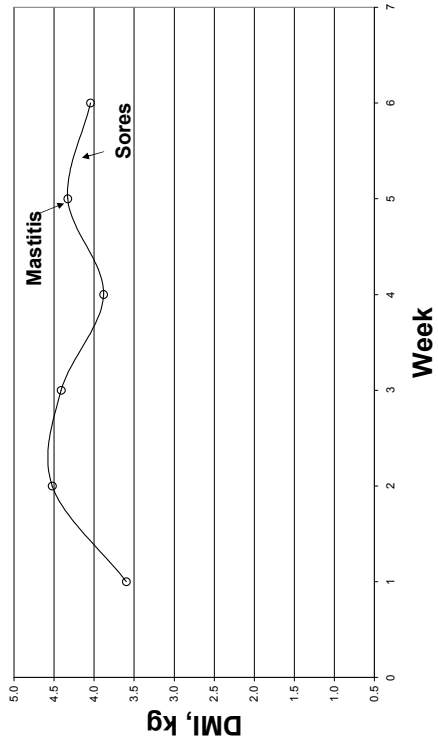
Ewe 21 - 35% FNDF



Ewe 17 - 35% FNDF



Ewe 21 - 35% FNDF



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