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Effects of dietary protein level and fish meal on growth and hormonal status of weaned dairy calves

Patricia Terrell Richardel
Louisiana State University and Agricultural and Mechanical College

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EFFECTS OF DIETARY PROTEIN LEVEL AND FISH MEAL ON GROWTH AND HORMONAL STATUS OF WEANED DAIRY CALVES

A Thesis

Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College
In partial fulfillment of the Requirements for the degree of Master of Science

in

The Interdepartmental Program in Animal and Dairy Sciences

by

Patricia Terrell Richardel
B.S., Louisiana State University, 2001
August, 2004
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ABSTRACT

Eight weaned Holstein steers approximately 4 mo of age (mean BW = 119.47 ± 2.65 kg) were used in a replicated 4 x 4 Latin square designed experiment to study the effects of protein source and level on performance and anabolic hormonal status of weaned dairy calves. Treatments consisted of two diets containing either 16 or 20 % crude protein (CP) with or without fish meal (FM). Experimental diets were corn silage based with soybean meal (SBM) as the source of ruminal degradable protein and FM as the source of ruminal undegradable protein. The animals were fed their respective diets twice daily at ad libitum levels during each 10-d adjustment period and 4-d sample collection period. Animals were housed in individual stalls for 10 d and in metabolism crates for 4 d for each experimental period. Feed intake was recorded daily. Total fecal and urine output were collected, weighed and subsampled during the 4-d sample collection period for laboratory analysis of nitrogen. On d 4 of the sample collection period, animals were fitted with indwelling jugular catheters. Blood samples were collected for 6 h at 15-min intervals for analysis of growth hormone (GH) and 30-min intervals for analysis of insulin (INS) and insulin-like growth factor-1 (IGF-1). An initial blood sample was taken at the beginning of the 6-h blood sample period for analysis of plasma urea nitrogen (PUN) and thyroxine (T₄). On d 14 of the each experimental period, body weight, wither height, hip height, and body length were measured. Treatment did not affect (P > 0.10) dry matter intake, any of the growth parameters measured, or nitrogen balance. Steers consuming diets containing 20% CP diets tended to have higher plasma INS concentrations (P = 0.07) whereas steers consuming SBM as the protein source tended to have higher mean GH levels (P= 0.07). Thyroxine, IGF-1,
and PUN concentrations were not effected by protein level or source (P > 0.10). It is concluded that feeding diets greater than 16% CP with or without FM does not improve performance in weaned dairy calves.
CHAPTER 1
INTRODUCTION

Emphasis on management of replacement heifers is becoming increasingly more important as the dairy industry continues to promote rapid growth to decrease time before first calving while still maintaining optimum production levels. Producers desire the replacement heifers to fully develop their lactation potential at the preferred age with minimal expense (James and Collins, 1992). Onset of puberty, however, is not determined strictly by age, but by body weight and size. Puberty is coupled with body weight that is usually about 55% of mature weight. Growth rates are dependant on the net energy available to the animal after the maintenance requirements are met (NRC, 2001). With the increasing emphasis on accelerated growth rates and earlier age at first calving, nutritional management of replacement heifers is extremely important.

Ruminants have the unique ability to convert feedstuffs not suitable for human consumption into products such as meat and milk through a synergistic relationship with the microbes of the rumen. The microbial population of the rumen is the factor that allows ruminants to make the conversion or poor quality feedstuffs to a high quality product and in turn, contribute to the human food supply. The rumen microbes can utilize feeds low in protein and energy for growth and reproduction. These microorganisms travel out of the rumen to the abomasum and then to the small intestines, where they are used by the ruminant as a protein supply. This microbial protein supply has an amino acid profile capable of meeting the maintenance requirements of most mature cattle (Church, 1988).
In young and growing animals, however, the microbial protein supply does not meet the requirements for the demand of amino acids (AA) essential for efficient muscle protein synthesis. In ruminant animals, the quality or quantity of the AA profile that enters the small intestine is determined predominantly by the AA profile of the microbial protein and not by the AA profile of the feedstuff. Changing the AA profile from one of primarily microbial origin to one which is a mixture of dietary and microbial AA would be more optimal for supporting growth (Merchen and Titgemeyer, 1992). The failure of microbial protein synthesis to provide for maximal productivity is demonstrated by post ruminal provision of AA or protein which improves nitrogen retention. Gill and Beever (1982) reported that a change from AA of primarily microbial origin to a mixture of microbial and dietary AA improved nitrogen balance in Holstein steer calves. Similarly, Davenport et al. (1990b) reported increased nitrogen retention in growing beef steers when casein was abomasally infused.

Growth is a multifaceted and highly integrated process. It entails many interactions between the genotype of the animal, the environment, the nutrition of the animal, and the hormonal status of the animal. Although maximum body size is genetically determined, the rate and extent of growth is impacted by nutritional and hormonal factors with the majority of control being through the actions of the endocrine system. The main hormone regulating growth is the adenohypophysial hormone, growth hormone (GH), otherwise known as somatotropin (Guyton and Hall, 2000). Many of the anabolic effects of GH however are modified by thyroxine (T₄) and insulin (INS), and mediated by insulin-like growth factor-1 (IGF-1).
Previous research has shown increases in anabolic hormone status in ruminants fed increased dietary crude protein levels (Davenport et al., 1995b). Post ruminal supply of AA via abomasal infusion has also resulted in increased INS, GH, and IGF-1 (Ragland-Gray et al., 1997; Guerino et al., 1991; Davenport et al., 1990b; Davenport et al., 1995b) in growing ruminants. Therefore, the objective of this study was to determine the effect of level and source of supplemental protein on growth and anabolic hormone status of weaned, growing Holstein steers as they transition into fully functional ruminants.
CHAPTER 2
REVIEW OF LITERATURE

Background

In young and growing ruminants, the microbial protein supply does not supply enough AA in quantity and quality to satisfy the demand for AA to synthesize muscle protein efficiently. Because the AA profile that enters the small intestine is determined predominantly by the AA profile of the microbial protein and not by the AA profile of the feedstuff, protein supplementation can change the AA profile from one of primarily microbial origin to one which is a mixture of dietary and microbial AA which would be more optimal for supporting growth (Merchen, and Titgemeyer, 1992). The deficiency in quality of the AA profile provided solely by microbial protein can be corrected by feeding supplemental protein sources. Amino acid absorption is too low to maintain maximum protein accretion when there is no protein supplementation (Barry et al., 1982). Without depressing forage intake, protein supplementation helps to correct the protein deficiency and alters the balance of protein and energy consumed (England and Gill, 1985). One method of protein supplementation is to increase the level of crude protein (CP) in the diet which increases the amount of AA that enters the rumen. A second method of protein supplementation is to provide rumen undegradable protein (RUP) in the diet, which increases the amount of AA reaching the abomasum.

Protein Supplementation Level

The nutritional status of growing animals is of utmost importance, especially the status of protein nutrition of the animal. Deficiency in protein can reduce the amount of gain in growing calves (NRC, 2001). Dawson et al. (1991) fed grass silage to young
Friesian steers and reported significantly increased growth rates in steers whose diet was supplemented with fish meal (FM) compared to the steers receiving only grass silage. Davenport et al. (1995b) reported abomasal flows of total nitrogen and bacterial nitrogen in growing lambs that increased linearly when the CP levels increased from 9% CP to 12% CP to 15% CP. They also reported an increase in flows of essential and nonessential AA. Hoffman et al. (2001) performed a growth study in which post pubertal heifers were fed diets containing 8, 11, 13 or 16% CP. These researchers observed linear increases in absorbed N as levels of CP increased. Bunting et al. (1989) observed similar results in Angus Heifer calves (234kg). These researchers observed greater nitrogen retention in calves with high protein intake (18.8% CP) compared to calves with low protein (10.2 % CP). Lammers and Heinrichs (2000) fed Holstein heifers a total mixed ration containing either a low (46:1), medium (54:1) or high (61:1) dietary CP to metabolizable energy ratio. These researchers reported an improvement in feed efficiency, increased ADG, and structural growth in heifers consuming diets with the high CP to metabolizable energy ratio.

**Rumen Undegradable Protein**

The deficiency in quality of the AA profile provided solely by microbial protein is corrected more so by feeding supplemental sources of low ruminal degradable protein or RUP. These types of protein sources resist degradation by the ruminal microbes and reach the abomasum basically unaltered (Merchen and Titgemeyer, 1992). Protein supplements that are high in RUP and are commonly used in ruminant diets are FM, feather meal, blood meal, corn gluten meal, distillers dried grains, distillers dried grains solubles, brewers dried grains, brewers wet grains, roasted soybeans, and heat treated
soybean meal (Santos et al., 1998). Because they resist degradation, the AA profile available for absorption is determined predominantly by the AA profile of the RUP supplement. Supplementation with RUP can improve animal gains by altering the protein to energy ratio. Beef steers fed ad libitum grass silage and supplemented with FM had increased average daily gains compared to those supplemented with barley (Veira et al., 1988). According to those researchers, improvement of the protein to energy ratio enhanced the animal’s ability to deposit protein and thus increased the growth of the beef steers. Bethard et al. (1997) reported that high RUP fed in the form of blood meal improved the feed efficiency of growing Holstein heifers as well as apparent total digestible nutrient efficiency. Donaldson et al. (1990) fed RUP in the form of FM and distiller’s dried grains to abomassally cannulated beef steers. These researchers reported greater dry matter intake and increased abomasal crude protein and nonammonia nitrogen flows compared to corn supplementation. This increase allowed an improvement in the AA balance available for absorption by the small intestine.

More importantly, RUP supplementation improves the quality and quantity of AA reaching the small intestine for absorption (Merchen and Titgemeyer, 1992). This change in AA profile could cause a growth response to occur. Tomlinson et al. (1997) reported growth responses when blood meal was added to the diets of Holstein heifers. In that study, heifers had improved feed efficiency as well as increased gains when RUP percentage increased in the diet. Zerbini and Polan (1985) observed improved average daily gains when FM was used as a source of RUP in diets of Holstein bull calves. Gill and Beever (1982) fed FM and ryegrass silage to Holstein steers and found an increased flow of AA into the small intestine with an increase in the amounts of specific AA
(methionine, lysine, arginine, and glutamine). These researchers also observed an increase in growth in the steers fed FM.

Fish meal is an excellent source of RUP and has been shown to cause an increase in growth when supplemented to the diets of growing ruminants. Addition of FM to formic-acid-treated grass silage fed to weaned steers resulted in significantly greater gains than silage alone or silage supplemented with barley (Veira et al. 1985). Additional work by Veira et al. (1988) resulted in increased average daily gains in beef steers fed ad libitum grass silage supplemented with FM compared to beef steers supplemented with barley. Addition of FM to the diets of beef calves increased average daily gain, feed efficiency and plasma AA profiles when compared to control diets that were supplemented with SBM only (Davenport et al., 1990b).

**Anabolic Hormones**

Changes in endocrinological status of the animal may be the means by which the supply of nutrients regulates protein metabolism and growth. A change in hormonal status caused from an increased or altered balance of AA absorbed could increase BW and protein gain (Barry et al., 1982). An increase in quality and quantity of AA supplied by AA bypassing degradation by the rumen can have an effect on hormonal influences of growth, especially the anabolic hormones INS, GH, and IGF-1 (Ragland-Gray et al., 1997).

Thyroxine is secreted by the thyroid gland and though not truly considered an anabolic hormone, it is essential for the growth promoting effect of GH. Thyroxine is required for both the secretion of GH and its actions. When T₄ concentrations drop to below physiological level, secretion of GH decreases, and in absence of T₄ growth is
severely retarded even if GH is present at physiological levels (Hadley, 2000).

Thyroxine affects nearly every tissue in the body, and its main effects involve overall control of metabolism of the animal. Thyroxine has a key role in the partitioning of nutrients to different body compartments. Its main functions include increasing the number of mitochondria and mitochondrial oxidative phosphorylation (increasing basal metabolic rate), stimulating the rapid uptake of AA into cells, increasing transcription and translation, and increasing the active transport of ions through the cell membranes (Hadley, 2000). Thyroid hormone also functions in carbohydrate metabolism by increasing cellular glucose uptake, glycolysis, and the absorption of glucose from the gastrointestinal tract. In lipid metabolism, T₄ increases mobilization of lipids from adipose tissue, free fatty acid concentration in plasma, and oxidation of free fatty acids in cells (Guyton and Hall, 2000).

Insulin is secreted by the Islets of Langerhans in the pancreas in response to energy abundance caused by a rise in blood glucose, fatty acids, or AA. Its actions in the body include stimulation of cellular glucose uptake, increased triglyceride synthesis, and increased incorporation of triglycerides into adipose tissue. More importantly, INS promotes protein synthesis and thus has a permissive effect on GH (Hadley, 2000; Guyton and Hall, 2000). It stimulates the cellular uptake of AA, increases transcription of DNA to form more mRNA, increases translation of the mRNA in the cells, diminishes gluconeogenesis by the liver, and inhibits protein catabolism (Guyton and Hall, 2000).

Growth hormone is necessary for somatic growth and is released by the adenohypophysis in response to a drop in blood glucose levels, increased levels of arginine and AA, or exercise. Growth hormone will cause growth in almost all cells
which have the ability to grow by causing an increase in cell size and division (Guyton and Hall, 2000). Though its actions involving growth are indirect and mediated by the somatomedins, it functions in the body by increasing blood glucose levels, promoting catabolism of adipose tissue, and promoting cartilage, long bone, and total body growth. Somatotropin is also active in the body by increasing protein synthesis by increasing cellular uptake of AA, enhancing translation and transcription of mRNA, and decreasing protein and AA catabolism (Hadley, 2000).

Insulin-like growth factor-1, previously referred to as somatomedin C, is considered the most important of somatomedins. It is released primarily from the liver in response to GH and mediates many of the biological actions of GH, mainly the effects on bone and skeletal muscle protein synthesis. It is believed to function by causing the mitogenic actions of GH on bone, cartilage, and muscle and stimulating muscle-protein synthesis. Because of its strong affinity for its plasma binding proteins, it prolongs the growth promoting effects of GH (Hadley, 2000).

**Effect of Post Ruminal Supply of Amino Acids on Hormonal Status**

Numerous studies have suggested that post ruminal supplementation of AA may influence the secretion of anabolic hormones. A study of the relationship between dietary nitrogen and IGF-1 in lambs by Davenport et al. (1995b) demonstrated that increasing dietary nitrogen intake correlated with high levels of serum IGF-1. That study provided data that suggested a relationship between IGF-1 and N status of growing ruminants may be dependant on post-ruminal availability of AA and not DM or OM. Arginine, a GH secretagogue, when provided via infusion through an abomasal cannula in beef heifers resulted in an increase in serum levels of GH (Davenport et al., 1990b). Guerino et al.
(1991) reported an increase in INS concentrations in beef steers when casein was abomassally infused. A post ruminal supply of AA affected hormonal status of growing Holstein steers abomassally infused with casein and arginine (Ragland-Gray et al., 1997). Those researchers reported increased GH concentrations when arginine was infused. They also reported increased concentrations of INS and IGF-1 combined with an increase in growth when casein was infused. Davenport et al. (1995b) also reported that abomasal arginine infusions resulted in increased GH concentrations when compared to control, whereas the infusion of casein resulted in an increase in serum levels of IGF-1 and INS serum levels. Moloney et al. (1998) studied the response of skeletal muscle IGF-1 to abomasal casein infusion in Holstein steers. Those researchers found that muscle IGF-1 mRNA significantly increased with the post ruminal supply of AA provided by infusion of casein.

Based on studies of post ruminal supply of AA and protein, it is thought that the greater flow of AA into the abomasum from dietary RUP could stimulate secretion of anabolic hormones and thus influence growth. There have been previous reports indicating that RUP supplementation can increase growth. Ragland-Gray et al. (1997) hypothesized that the increased flow of AA to the abomasum could stimulate I release because of the greater amount of AA absorbed into the blood. Greater flow of AA to the abomasum from RUP could also increase the amount of arginine absorption which may stimulate GH release from the hypothalamus and subsequently IGF-1 release from the liver (Ragland, 1993). The provision of supplemental protein in the diets of growing heifers may improve growth and development by influencing the anabolic hormones which would be a useful tool in dairy cattle management.
CHAPTER 3
MATERIALS AND METHODS

Animals and Dietary Treatments.

Eight weaned Holstein steers (119.47 ± 2.65 kg; 4mo) were utilized in an 8-wk experiment to determine the effects of level of dietary crude protein and the effects of addition of RUP on growth and anabolic hormone status. The eight steers utilized in this study were born at the Southeast Research Station in Franklinton, LA between November, 2002, and December, 2002. All steers were transported to the LSU Agricultural Center Research and Teaching Farm in Baton Rouge, LA one month prior to the initiation of the experiment and housed there for the duration of the experiment. The experimental protocol was approved by the Institutional Animal Care and Use Committee of the LSU Agricultural Center.

The eight steers were used in a replicated 4 x 4 Latin square designed experiment. The experimental diets were corn-silage based, with soybean meal (SBM) as the source of ruminal degradable protein and Menhaden FM (SeaLac™, Omega Protein™ Inc., Hammond, LA) as the source of RUP. Treatments consisted of diets formulated to contain 1)16% CP with SBM 2) 20% CP with SBM 3) 16% CP with FM, and 4) 20% CP with FM. Proportions of each ingredient for the experimental diets are listed in Table 1. Chemical analysis of the diets is listed in Table 2. The individual diets were mixed daily and fed as a total mixed ration. The animals were fed their respective diets twice per day at ad libitum levels during each 14-d experimental period, which included a 10-d adjustment period and a 4-d sample collection period. Water was
Table 1
Composition of the experimental diets.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Soybean meal</th>
<th>Fish meal</th>
<th>Soybean meal</th>
<th>Fish meal</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of DM</td>
<td>16 % CP</td>
<td>20 % CP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corn silage</td>
<td>32.91</td>
<td>33.08</td>
<td>32.61</td>
<td>33.80</td>
</tr>
<tr>
<td>Bermuda grass hay</td>
<td>20.37</td>
<td>20.48</td>
<td>20.18</td>
<td>20.30</td>
</tr>
<tr>
<td>Corn grain</td>
<td>26.53</td>
<td>26.66</td>
<td>19.94</td>
<td>20.06</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>18.32</td>
<td>16.84</td>
<td>25.44</td>
<td>23.07</td>
</tr>
<tr>
<td>Fish meal</td>
<td>0.00</td>
<td>1.09</td>
<td>0.00</td>
<td>1.93</td>
</tr>
<tr>
<td>Mineral premix(^1)</td>
<td>1.56</td>
<td>1.54</td>
<td>1.52</td>
<td>1.53</td>
</tr>
<tr>
<td>Lasalocid(^2)</td>
<td>0.31</td>
<td>0.31</td>
<td>0.31</td>
<td>0.31</td>
</tr>
</tbody>
</table>

\(^1\) McNess Calf Premix B: 17.80% Ca, 4.5% P, 1.0% Mg, 0.60% K, 7.80% Na, 12.20%
Cl, 8.00% S, 44 ppm Co, 875 ppm Cu, 2530 ppm Fe, 57 ppm I, 2860 ppm Mn,
19.5 ppm Se, 2879 ppm Zn, 211 KIU/lb Vitamin A, 21.6 KIU/lb Vitamin D, 380
KIU/lb Vitamin E.

\(^2\) Bovatec\(^{TM}\)
Table 2
Least squares means for the chemical analysis of experimental diets.

<table>
<thead>
<tr>
<th>Item</th>
<th>16% CP</th>
<th>20% CP</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Soybean meal</td>
<td>Fish meal</td>
<td>Soybean meal</td>
</tr>
<tr>
<td>Dry matter, %</td>
<td>56.1</td>
<td>55.5</td>
<td>56.4</td>
</tr>
<tr>
<td></td>
<td>% of DM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Organic matter, %</td>
<td>89.09</td>
<td>88.82</td>
<td>88.15</td>
</tr>
<tr>
<td>CP(^1)</td>
<td>16.52</td>
<td>16.47</td>
<td>19.56</td>
</tr>
<tr>
<td>ADIN(^2)</td>
<td>0.23</td>
<td>0.23</td>
<td>0.26</td>
</tr>
<tr>
<td>Bound CP(^3)</td>
<td>1.46</td>
<td>1.44</td>
<td>1.6</td>
</tr>
<tr>
<td>Available CP(^4)</td>
<td>15.06</td>
<td>15.03</td>
<td>17.96</td>
</tr>
<tr>
<td>NDF</td>
<td>36.39</td>
<td>36.54</td>
<td>36.24</td>
</tr>
<tr>
<td>ADF</td>
<td>30.61</td>
<td>29.83</td>
<td>34.46</td>
</tr>
</tbody>
</table>

\(^1\)CP = N x 6.25.
\(^2\)ADIN = acid detergent insoluble nitrogen
\(^3\)Bound CP = ADIN x 6.25.
\(^4\)Available CP = CP – Bound CP.
available *ad libitum*. Animals were housed in individual pens (6.09 m x 4.88 m) for 10 d and in metabolism crates (152.4 cm x 76.2 cm x 121.92 cm) for 4 d during each period.

**Sample Collection.**

On d 14 of each experimental period body weight, wither height, hip height, and body length were measured. The height and length measurements were collected as previously described by Larson et al. (1977).

On d 10 to 13 total fecal output was collected, weighed, and a 5% subsample was reserved for laboratory analysis. Subsamples were dried to a constant weight at 55º C, compositied by steer and period, and ground through a Wiley mill (2mm screen). Dry matter, ash, and N were determined by AOAC (1980) procedures. NDF and ADF were determined using an Ankom 200 fiber analyzer (Ankom Technology; Macedon, NY).

Urine was collected daily on d 10 to 13 and volume was measured. Urine was preserved between collections in 6 N HCl (100 ml). Aliquots (10%) were composited by steer and period and frozen until analyzed for N as described above.

Diet and ort grab samples from the individual steers were collected daily during each 14-d period. Samples were composited by steer and period with composites being divided by adjustment or collection days. Individual feed ingredients were sampled weekly. All feed, diet and ort samples were dried to a constant weight, ground, subsampled and analyzed for DM, ash, and N by methods previously described.

On d 14 of each experimental period, animals were fitted with 14G x 5.1cm indwelling jugular catheters (Baxter Healthcare Corporation, Deerfield, IL) and allowed to rest for 1 h. A 6% sodium citrate solution was used to flush catheters before and between sampling. A blood sample was collected from each steer for plasma urea
nitrogen and T₄ analysis approximately 1 h after catherization. Blood samples were then collected from each steer at 15-min intervals for 6 h for analysis of GH. Concurrently, additional blood samples from each steer were collected at 30-min intervals for analysis of IGF-I and INS. All blood samples were placed into 7-mL evacuated tubes containing potassium oxalate and sodium fluoride (Kendall Medical, St. Louis, MO), chilled, and then centrifuged at 1,876 x g for 20 min. The plasma was removed and stored at -20ºC until analysis.

**Laboratory Methods**

Plasma INS concentrations were measured using commercial radioimmunoassay kits (Coat-A-Count; Diagnostic Products Corporation, Los Angeles, CA). The inter- and intraassay coefficients of variation were 3.0% and 5.6%, respectively. Plasma T₄ was also measured using commercial radioimmunoassay kits (T₄ Monoclonal Solid Phase Radioimmunoassay; ICN Pharmaceuticals, Inc., Costa Mesa, CA). The inter- and intraassay coefficients of variation were 2.1% and 2.1%, respectively. Plasma was analyzed for urea nitrogen concentrations using commercial spectrophotometric kits, (Urea Nitrogen (BUN) Berthelot/ Colorimetric; Pointe Scientific, Inc., Lincoln Park, MI). The inter- and intraassay coefficients of variation were 4.7% and 4.7%, respectively.

Plasma concentrations of GH were measured via radioimmunoassay procedures as described by Granger et al. (1989). The inter- and intraassay coefficients of variation were 3.1% and 6.0%, respectively. Plasma concentrations of IGF-1 were measured using radioimmunoassay procedure as described by Sticker et al. (1995) as modified for cattle. The inter- and intraassay coefficients of variation were 2.3% and 4.3%, respectively.
**Statistical Analyses**

Data for growth, intake, digestibility, diet composition, N balance, PUN, and T₄ were analyzed as a replicated Latin square designed experiment using a mixed model (Littell et al., 1998). The model included terms for fixed effects of steer, period, protein level, protein source, and the interactions of protein source and level. Square and the interaction of square with all fixed effects were included as random terms. Data for IGF-1, INS, and GH were analyzed as repeated measures within the Latin square using a mixed model (Littell et al., 1998). The model included terms for fixed effects of steer, period, source of protein, level of protein, and the interaction of source and level of protein. Square, the interaction of square with all fixed effects and steer nested within the interaction of source of protein, level of protein, and period were included as random effects. Time and the interaction of time with all fixed effects were included as a repeated term that was assumed to have a constant covariance structure. All data are presented as least squares means. All calculations were completed using SAS (SAS Institute Inc., 1990).
CHAPTER 4
RESULTS AND DISCUSSION

Performance Data

Least squares means for average daily DMI and daily increases in growth parameters are listed in Table 3. There was no effect (P > 0.05) of dietary protein level or addition of FM on mean daily DMI. Davenport et al. (1995b) reported similar results in Suffolk wether lambs consuming 9, 12, and 15% CP. Davenport et al. (1990a) also reported no difference in DMI in crossbred beef calves fed *ad libitum* corn silage supplemented with ground SBM with or without FM and or rumen-protected lysine. In contrast, Donaldson et al. (1991) observed an increase in DMI in 12 month old Hereford steers grazing annual ryegrass supplemented with FM and distiller’s dried grains compared to animals supplemented with corn. England and Gill (1985) also observed an increase in DMI in British Friesian steers fed ryegrass silage supplemented with FM compared to steers supplemented with sucrose.

There was no effect (P > 0.05) of protein level or source on ADG, or gains in wither height, hip height, or body length. Because ADG across all treatments was above the NRC (2001) recommendation for Holstein steers at this age, these results indicate that all of the diets provided adequate amounts of metabolizable protein for growth throughout the periods. Neary et al. (1995) reported no influence of level of CP fed on ADG regardless of FM supplementation. Similar results were detected by Tomlinson et al. (1997) who reported no significant difference in growth, wither height, or heart girth between Holstein heifers fed 31, 43, 50, and 55% of the dietary N as RUP (blood meal).
Table 3

Least squares means of average daily dry matter intake, body weight gain, and skeletal growth in steers fed diets containing 16% or 20 % CP with or without FM.

<table>
<thead>
<tr>
<th></th>
<th>16% CP</th>
<th>20% CP</th>
<th>P =</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Soybean meal</td>
<td>Fish meal</td>
<td>Soybean meal</td>
</tr>
<tr>
<td>DMI (kg/d)</td>
<td>8.11</td>
<td>8.01</td>
<td>8.11</td>
</tr>
<tr>
<td>ADG (kg/d)</td>
<td>1.08</td>
<td>0.90</td>
<td>1.03</td>
</tr>
<tr>
<td>Wither height (cm/d)</td>
<td>0.25</td>
<td>0.19</td>
<td>0.21</td>
</tr>
<tr>
<td>Hip height (cm/d)</td>
<td>0.24</td>
<td>0.18</td>
<td>0.21</td>
</tr>
<tr>
<td>Body length (cm/d)</td>
<td>0.36</td>
<td>0.27</td>
<td>0.26</td>
</tr>
</tbody>
</table>
Coomer et al. (1993) also reported no effect of increasing RUP supplementation on ADG or DMI in Holstein steers or heifers. Others have reported conflicting results. Pirlo et al. (1997) observed an effect on ADG in Italian Friesian heifers fed 110% of the 1989 NRC recommended amount of CP. Huntington et al. (2001) reported similar results with Angus steers which had an increase ADG when supplemental protein increased from 100 g/d to 200 g/d. Hoffman et al. (2001), however, performed a growth study in which post pubertal heifers were fed diets containing 8, 11, 13 or 16% CP. Those researchers reported linear increases in wither height gain in heifers consuming increasing amounts of CP. Numerous studies have shown that supplementation with RUP resulted in increased growth and ADG of beef and dairy cattle (Davenport, et al. 1990a; Dawson, et al., 1991; Veira, et al., 1988; Zerbini and Polan, 1985).

Least squares mean for digestibilities of dietary components are listed in Table 4. There was no difference (P > 0.05) between the experimental diets in the digestibility of DM, OM, NDF, ADF, or CP. In contrast, Donaldson et al. (1991) observed an increase in total tract DM digestion in 12-mo old Hereford steers grazing annual ryegrass supplemented with FM and distiller’s dried grains compared to animals supplemented with corn. Research involving RUP in the form of FM has also shown contrasting results to the data of this experiment. England and Gill (1985) reported increases in OM and N digestibility in British Friesian steers fed ryegrass silage supplemented with FM compared to steers supplemented with sucrose.

**Nitrogen Balance and Hormone Data**

Nitrogen balance data for the steers are presented in Table 5. There was no effect (P > 0.05) of protein level or FM supplementation on nitrogen balance or PUN.
Table 4

Least square means of digestibilities for experimental diets containing 16% or 20 % CP with or without fish meal.

<table>
<thead>
<tr>
<th></th>
<th>16% CP</th>
<th>20% CP</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Soybean meal</td>
<td>Fish meal</td>
<td>Soybean meal</td>
</tr>
<tr>
<td>DM, %</td>
<td>71.77</td>
<td>74.13</td>
<td>74.48</td>
</tr>
<tr>
<td>OM, %</td>
<td>71.66</td>
<td>73.72</td>
<td>74.58</td>
</tr>
<tr>
<td>CP, %</td>
<td>78.13</td>
<td>79.07</td>
<td>81.79</td>
</tr>
<tr>
<td>NDF, %</td>
<td>54.94</td>
<td>52.30</td>
<td>51.77</td>
</tr>
<tr>
<td>ADF, %</td>
<td>68.30</td>
<td>66.66</td>
<td>70.53</td>
</tr>
<tr>
<td></td>
<td>16% CP</td>
<td>20% CP</td>
<td>SEM</td>
</tr>
<tr>
<td>---------------------</td>
<td>---------</td>
<td>---------</td>
<td>-------</td>
</tr>
<tr>
<td>N intake, g/d</td>
<td>214.37</td>
<td>253.87</td>
<td>15.364</td>
</tr>
<tr>
<td>Fecal N, %</td>
<td>2.66</td>
<td>2.63</td>
<td>0.065</td>
</tr>
<tr>
<td>Urinary N, %</td>
<td>0.57</td>
<td>0.82</td>
<td>0.110</td>
</tr>
<tr>
<td>N balance, g/d</td>
<td>147.40</td>
<td>174.84</td>
<td>16.036</td>
</tr>
<tr>
<td>N absorbed, g/d</td>
<td>176.13</td>
<td>219.89</td>
<td>13.528</td>
</tr>
<tr>
<td>N digestibility, %</td>
<td>78.13</td>
<td>81.79</td>
<td>1.649</td>
</tr>
<tr>
<td>PUN, mg/dL</td>
<td>15.55</td>
<td>21.61</td>
<td>2.016</td>
</tr>
</tbody>
</table>
concentrations of steers. There was, however, a tendency for steers consuming 20% CP diets to have higher levels of nitrogen intake (P = 0.10) and nitrogen absorbed (P = 0.09) than steers consuming 16% CP diets. Davenport et al. (1990a) reported no effect of treatment on PUN concentrations in crossbred beef calves fed ad libitum corn silage supplemented with ground SBM with or without FM and or rumen-protected lysine. In contrast to this experiment’s data, Davenport et al. (1995b) observed an increase in nitrogen retention and blood urea nitrogen when dietary nitrogen increased in Suffolk wether lambs fed increasing amounts of dietary nitrogen (9, 12, or 15% CP). Bunting et al. (1989) reported an increase in nitrogen retention in Angus heifer calves with high protein intake (18.8% CP) compared to calves with low protein (10.2% CP). Hoffman et al. (2001) observed linear increases in absorbed N in pubertal heifers fed increasing levels of CP (8, 11, 13 or 16% CP). Gabler and Heinrichs (2003) reported plasma urea nitrogen concentrations increased in Holstein heifers fed increasing amounts of CP (11.9%, 16.7%, 18.1%, and 20.1%).

Least squares means for plasma concentrations of INS, T₄, GH, and IGF-1 are reported in Table 6. There was no effect of protein level or source on T₄ concentrations (P > 0.05). Insulin was not affected (P > 0.05) by the inclusion of FM as a source of RUP. Diets containing 20% CP, however, tended to have higher circulating INS concentrations (P = 0.0762) compared to diets with 16% CP. There was a main effect of time on INS concentration (P < 0.01) (Figure 1). Gerrits et al. (1998) reported an increase in INS plasma concentrations when crossbred beef calves consumed increasing protein intake. These researchers also reported an increase in T₄ plasma concentrations.
Table 6

Least squares mean for plasma concentrations of insulin, thyroxine, growth hormone, and insulin-like growth hormone for steers fed diets containing 16% or 20% CP with or without fish meal.

<table>
<thead>
<tr>
<th></th>
<th>16% CP</th>
<th>20% CP</th>
<th>P =</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Soybean Meal</td>
<td>Fish Meal</td>
<td>Soybean Meal</td>
</tr>
<tr>
<td>Plasma Insulin (µIU/mL)</td>
<td>13.99</td>
<td>11.57</td>
<td>14.92</td>
</tr>
<tr>
<td>Plasma Thyroxine (µg/dL)</td>
<td>7.51</td>
<td>8.07</td>
<td>8.22</td>
</tr>
<tr>
<td>Plasma Growth Hormone (ng/mL)</td>
<td>15.63</td>
<td>13.54</td>
<td>14.04</td>
</tr>
<tr>
<td>Plasma IGF-1 (ng/mL)</td>
<td>189.60</td>
<td>177.86</td>
<td>199.30</td>
</tr>
</tbody>
</table>
Figure 1. Plasma insulin concentrations during a 6 hour period in Holstein steers fed diets containing 16% CP SBM, 20% CP SBM, 16% CP FM, or 20% CP FM. Main effect of time (P < 0.01; SEM = 2.139 ).
Increases in INS concentrations have also been reported when casein was abomasally infused in growing ruminants (Guerino et al., 1991; Davenport et al., 1995b; Ragland-Gray et al., 1997).

Plasma growth hormone concentrations were not affected by dietary protein level (P > 0.05), although steers fed SBM tended to have greater mean GH (P = 0.0741). Previous research has shown that post ruminal supply of amino acids resulted in increased levels of GH. Davenport et al. (1995a) reported increased mean plasma GH concentrations in wether lambs fed rumen-protected AA (ornithine and arginine) supplement when compared to wether lambs fed no supplements. Increases in GH have also been reported when arginine was infused through an abomasal cannula in cattle (Davenport et al., 1990b, Davenport et al. 1995b, Ragland-Gray et al., 1997). The results of plasma GH concentrations during the 6-h sampling time periods are presented in Figure 2. There was a main effect of time on GH concentrations (P < 0.01). It has been previously shown that GH is secreted in a pulsatile manner during the day (Gluckman et al., 1987).

Neither level nor source of protein had an effect on mean plasma IGF-1 concentrations (P > 0.05). Results from this study disagree with previous research in which increased CP and RUP levels increased IGF-1 in growing ruminants. Davenport et al. (1995b) reported increased mean IGF-1 concentrations in lambs consuming increasing amounts of N (9, 12, or 15% CP) and demonstrated that increasing dietary nitrogen intake correlated with high levels of serum IGF-1. Davenport et al. (1995a) also observed increased serum IGF-1 concentrations in lambs fed rumen-protected AA. Ragland-Gray
Figure 2. Plasma growth hormone concentrations during a 6 hour period in Holstein steers fed diets containing 16% CP SBM, 20% CP SBM, 16% CP FM, or 20% CP FM. Main effect of time (P < 0.01; SEM = 2.384).
Figure 3. Plasma insulin-like growth factor-I concentrations during a 6 hour period in Holstein steers fed diets containing 16% CP SBM, 20% CP SBM, 16% CP FM, or 20% CP FM. No significance of time (P > 0.05; SEM = 28.306)
et al. (1997) and Davenport et al. (1995b) reported increased concentrations of IGF-1 combined with an increase in growth when casein was infused abomasally in cattle. Least squares means of IGF-1 over time are shown in Figure 3. There were no effects of time on IGF-1 concentrations, thus supporting previous research that IGF-1 concentrations remain stable throughout the day (Gluckman et al., 1987).
CHAPTER 5

SUMMARY AND CONCLUSION

Summary

The objective of this study was to determine the effect of level and source of supplemental protein on growth and anabolic hormone status of weaned, growing Holstein steers as they transition into fully functional ruminants.

Eight weaned Holstein steers (119.47 ± 2.65 kg; 4mo) were utilized in an eight-week, replicated 4 x 4 Latin square designed experiment to determine the effects of level of dietary crude protein and the effects of addition of RUP on growth and anabolic hormone status. Experimental diets were corn-silage based, with SBM as the source of rumen degradable protein and Menhaden FM (SeaLac™, Omega Protein™ Inc., Hammond, LA) as a source of RUP. Treatments consisted of diets formulated to contain 1) 16% CP with SBM (16% CP SBM) 2) 20% CP with SBM (20% CP SBM) 3) 16% CP with FM (16% CP FM), and 4) 20% CP with FM (20% CP FM). The individual diets were mixed daily and fed as a total mixed ration with each animal receiving its respective diets twice per day at ad libitum levels during each 14-d experimental period, which included a 10-d adjustment period and a 4-d sample collection period. Animals were housed in individual pens (6.09 m x 4.88 m) for 10 d and in metabolism crates (152.4 cm x 76.2 cm x 121.92 cm) for 4 d for each treatment. Total fecal and urine output were collected weighed and subsampled for nitrogen analysis during the 4-d collection period. On d 14 the steers were fitted with indwelling jugular catheters. Blood samples were collected at 15-min interval for 6 h for analysis of GH and at 30-min intervals for analysis of Ins. One additional blood sample was collected at the 0-min of the 6-h bleeding period.
for analysis of T₄ and PUN. On d 14 of each experimental period body weight, wither height, hip height, and body length were measured

Treatment did not affect DMI (P > 0.05) nor did it effect any of the growth parameters measured (P > 0.05). These results indicate that all of the diets provided adequate amounts of metabolizable protein for growth throughout the periods. There were also no significant differences between the experimental diets in the digestibility of DM, OM, NDF, ADF, or CP (P > 0.05). There were no effects (P > 0.05) of protein level or of FM supplementation on the nitrogen balance or the PUN concentrations of steers.

There were no effects on either INS or T₄ plasma concentrations (P > 0.05) with the inclusion of FM as a source of RUP. However, diets containing 20% CP tended to have higher circulating INS concentrations (P=0.07) compared to diets with 16% CP. Plasma GH concentrations were not affected by dietary protein level (P > 0.05), although steers fed SBM tended to have greater mean GH plasma concentrations (P = 0.07).

**Conclusion**

These data suggest that feeding diets greater than 16% CP with or without fish meal does not improve performance in weaned dairy calves. Because the researchers of this experiment were concerned about the palatability of FM to weaned dairy calves, the amount of FM included in the experimental diets did not increase the RUP value. The RUP values as a percent of CP for 16 and 20% CP with SBM as calculated by the NRC Ration Calculator (2001) were 36.3% and 36.5% respectively. The RUP values as a percent of CP for 16 and 20% CP with FM as calculated by the NRC Ration Calculator (2001) were 37.5 % and 38.3% respectively. Because the RUP values were so similar throughout the diets, there may not have been enough RUP to elicit responses previously
observed by other researchers. More research is needed to determine if inclusion of FM or other sources of RUP in diets of growing ruminants does, in fact, impact growth and hormonal status.
REFERENCES


Ragland, K. 1993. Supplemental protein alters digestion, nitrogen balance, and hormonal status in growing ruminants fed winter grass forages. A dissertation submitted to University of Georgia for PhD.


APPENDIX A

PLASMA INSULIN RADIOIMMUNOASSAY

(REF: Diagnostic Products Corporation, Coat-A-Count Insulin Kit, Catalog Number TKN5, Diagnostic Products Corporation, 5700 West 96th Street, Los Angeles, CA 90045-5597)

Principle
This kit is composed of a solid-phase radioimmunoassay with $^{125}$I labeled insulin competing for a fixed time with a non-labeled insulin in the sample for sites on an insulin-specific antibody. The antibody is immobilized to the tube wall. Decanting the supernatant terminates the competition and isolates the antibody-bound fraction of the radiolabeled insulin. A gamma counter is then used to yield a number which, by means of a calibration curve conversion table, measures the insulin present in the sample.

Radioimmunoassay Procedure

1. **Plain Tubes:** Label four plain (uncoated) 12 x 75 mm polypropylene tubes T (total counts) and NSB (nonspecific binding) in duplicate.
   **Coated tubes:** Label fourteen Insulin AB-coated Tubes A (maximum binding) and B through G in duplicate. Label AB-coated tubes, also in duplicate, for controls and test samples.
2. Pipette 200µL of the zero calibrator A into the NSB and A tubes, and 200µL of each remaining calibrator, control and test sample into the tubes prepared. Pipette directly to the bottom of the tube.
3. Add 1.0mL of $^{125}$I Insulin to every tube. Vortex.
4. Incubate for 18-24 hours at room temperature (15-28°C).
5. Decant thoroughly.
6. Count for one minute in a gamma counter.

Standard Curve

<table>
<thead>
<tr>
<th>Calibrator</th>
<th>Approximate µIU/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (MB)</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>5</td>
</tr>
<tr>
<td>C</td>
<td>15</td>
</tr>
<tr>
<td>D</td>
<td>50</td>
</tr>
<tr>
<td>E</td>
<td>100</td>
</tr>
<tr>
<td>F</td>
<td>200</td>
</tr>
<tr>
<td>G</td>
<td>400</td>
</tr>
</tbody>
</table>
Preparation of Reagent Solutions

1. **$^{125}$Insulin:** Add 100mL of distilled or deionized water to each vial of concentrated iodinated insulin. Mix by gentle inversion. Store refrigerated. Stable at 2-8°C for 30 days after preparation.

2. **Insulin Calibrators:** At least 30 minutes before use, reconstitute the zero calibrator A, with 6.0mL of distilled or deionized water. Reconstitute the remaining calibrators B through G with 3.0mL each of distilled or deionized water. Use volumetric pipettes and mix gently by swirling. Store frozen. Stable at -20°C for 30 days after reconstitution.

Calculations

1. Average duplicate counts for Total Count tubes, NSB tubes, Maximum Binding and remaining tubes.
2. Subtract the average NSB counts from each average count.
3. Calculate the percentage of tracer bound \[ \frac{(\text{Maximum Binding Counts} \times 100)}{\text{Total}} \]. This should be 35-50%.
4. Calculate the percentage of maximum binding \[ \frac{\%B}{B_o} = \frac{(\text{Sample or Standard} \times 100)}{\text{Maximum Binding}} \].
5. Plot the \( \frac{\%B}{B_o} \) for each standard on the y-axis and the known concentration of the standard on the x-axis.
6. Construct the reference curve by joining the points with a smooth curve.
7. Determine the pg/mL of insulin in the unknown samples by interpolation of the reference curve.
APPENDIX B

PLASMA THYROXINE RADIOIMMUNOASSAY

(REF: T4 Monoclonal Solid Phase Radioimmunoassay; Catalog Number 06B56100-R6, ICN Pharmaceuticals, Inc., Diagnostics Division, 13 Mountain View Avenue, Costa Mesa, CA 10962-1294)

Principle

In radioimmunoassay (RIA), the antibody used should have an equal affinity for the standard and the analyte which is present in the serum. The unlabeled analyte competes with labeled analyte for the limited number of available antibody sites thereby reducing the amount of labeled analyte bound to antibody. The level of radioactivity bound is, therefore, inversely related to the concentration of the analyte in the sample or standard. After an adequate incubation period, the bound and free fractions are separated and the radioactivity quantitated. The ICN Pharmaceuticals [125I] T₄ (Thyroxine) Solid Phase Radioimmunoassay used tubes coated with Monoclonal T₄ antibody.

Radioimmunoassay Procedure

1. Number an appropriate number of antibody coated tubes for the standard curve and clinical samples.
2. Add 25µL of the T₄ Standards and samples to the appropriate tubes.
3. Add 1.0 mL of ¹²⁵I T₄ Tracer to each tube. Vortex.
4. Incubate for 1 hour at room temperature (15-28°C).
5. Decant thoroughly. Allow the tubes to drain from each tube for at least one minute. Tap and blot any residual liquid from the tube rims.
6. Count the radioactivity bound to the coated tubes in sequence for one minute with a gamma counter.

Standard Curve

<table>
<thead>
<tr>
<th>Standard</th>
<th>Approximate T₄ µg/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>2</td>
</tr>
<tr>
<td>C</td>
<td>4</td>
</tr>
<tr>
<td>D</td>
<td>8</td>
</tr>
<tr>
<td>E</td>
<td>12</td>
</tr>
<tr>
<td>F</td>
<td>20</td>
</tr>
</tbody>
</table>
Calculations

1. Average duplicate counts for Total Count tubes, NSB tubes, Maximum Binding and remaining tubes.
2. Subtract the average NSB counts from each average count.
3. Calculate the percentage of tracer bound \[\frac{\text{Maximum Binding Counts}}{\text{Total}} \times 100\]. This should be 35-50%.
4. Calculate the percentage of maximum binding \[\%B/B_o = \frac{\text{Sample or Standard}}{\text{Maximum Binding}} \times 100\].
5. Plot the \(\%B/B_o\) for each standard on the y-axis and the known concentration of the standard on the x-axis.
6. Construct the reference curve by joining the points with a smooth curve.
7. Determine the pg/mL of insulin in the unknown samples by interpolation of the reference curve.
APPENDIX C

PLASMA UREA NITROGEN COLORIMETRIC ASSAY

(REF: Urea Nitrogen (BUN) (Berthelot/Colorimetric) Assay; Pointe Scientific, Inc., 1025 John A Papalas, Lincoln Park, MI 48146)

**Principle**

The Berthelot reaction, in which ammonia reacts with hyperchlorite, phenol, a catalyst, and alkali to produce a stable blue complex (indophenol) has been known for over 100 years but only relatively recently used in a method for serum urea. The use of sodium nitroferricyanide was introduced in 1962 and the substitution of salicylate for phenol was introduced in 1967.

This procedure is based upon a modified Berthelot reaction wherein urease hydrolyses urea to ammonia and carbamic acid. Carbamic acid spontaneously decomposes into ammonia and carbon dioxide. Ammonia reacts with salicylate, nitroferricyanide and an alkaline solution of hypochlorite to yield a blue-green chromophore which is measured photometrically and is proportional to the amount of urea in the sample.

**Procedure**

1. Turn spectrophotometer on to warm up (~30 min.). Label cuvettes UNKNOWN, CONTROL, STANDARD, and BLANK in duplicate.
2. Transfer 0.5mL of COLOR RGT to vials labeled: UNKNOWN, CONTROL, STANDARD, BLANK.
3. Add 0.010mL (10µL) of sample in to corresponding vial.
4. Add 0.5 mL of ENZYME RGT to all vials, mix gently, and incubate for ten minutes at room temperature (26°C)
5. Add 2.0mL of BASE RGT, mix and incubate at room temperature (26°C) for ten minutes.
6. Set the absorbance reading to 0.00 against BLANK.
7. Set wavelength at 630nm.
8. Read the STANDARD cuvettes and enter the standard value.
9. Read and record the absorbances of all the cuvettes.

**Calculation**

Sample Urea Nitrogen = \( \frac{\text{Abs of Sample} \times C \text{ Standard mg/dL}}{\text{Abs Standard}} = C \text{ sample} \)
### APPENDIX D

**PLASMA GROWTH HORMONE RADIOIMMUNOASSAY**

**Volumes of reagents to pipette:**

1. **Anti-oGH**  
   - 200µL

2. **1:105 NRS #3**  
   - 200µL (1:42 + 50% horse serum) already made or add 200µL 1:42 NRS +100µL LE per NRS tube

3. **1:45 pARGG #3**  
   - 200µL

4. **125I-bGH**  
   - 200µL or amount on bottle

5. **PBSG**  
   - 200µL
   - *this volume changes for different standard tubes*

6. **LE2**  
   - 200µL

7. **HB35ng/mL**  
   - 200µL

8. **Sample**  
   - 200µL  
   - *If this volume change, make up to 200µL With PBSG and change LE & HB accordingly*

9. **STD (bgh 100ng/mL)**  
   - 1.6, 3.2, 6.2, 12.4, 25, 50, 100, 200µL

10. **STD (PBSG)**  
    - 198.4, 196.8, 193.8, 187.6, 175, 150, 100, 0µL

**What Goes in Each Tube**

1. **TC**  
   - 125I-bGH (HH)

2. **NRS**  
   - PBSG (200µL) + #3 1:105 NRS + HH + 1:45 (4X)pARGG

3. **BC**  
   - PBSG (200µL) + Anti-oGH (1:600) + HH + 1:45 (4X)pARGG

4. **STD**  
   - PBSG (follow the curve) + bGH 35ng/mL + Anti-oGH + HH + pARGG

5. **LE**  
   - LE + Anti-oGH + HH + pARGG

6. **HB**  
   - HB + Anti-oGH + HH + pARGG

7. **Sample**  
   - Sample + Anti-oGH + HH + pARGG
APPENDIX E

PLASMA INSULIN-LIKE GROWTH FACTOR-1 RADIOIMMUNOASSAY

Day 1 – Sample Preparation

1. Create a protocol for the sample extraction in Excel:
   a. The samples are in single. Put sample number (1,2,3…end) in column A, put sample ID in column B
   b. Samples 1-3 are to be LE2, Low Bovine, and Blank
   c. Follow with your samples
   d. End with LE2, Low Bovine, and Blank (there will be a total of N+6 tubes)
2. Label tubes for sample extraction (1, 2, … N+6)
3. Add 300µL of glycine-HCl (pH =3.2) mixture to all tubes.
4. Pipette 200 µL of each sample INTO the glycine-HCl mixture in the appropriate tube (do NOT dribble down the side of the tube).
5. When all sample are pipetted, vortex the tubes gently (but thoroughly mix) for 5 seconds.
6. Cover, label, and place in fridge for 24 hours.

Day 2 – Sample Neutralization and Assay Set-up

7. To each sample extraction tube, add 2.6 mL of PBS-EDTA.
8. Vortex thoroughly (several repetitions of 3-5 seconds each).
9. Create an assay protocol per normal (2TC, 3 NSB, 3BC, 8 STD), sample 1 in duplicate, sample 2, in duplicate,… sample N +6 in duplicate, 3 NSB, 3 BC, 8 STD, 2 TC).
10. Label assay tubes (total number will be 2(N+6) + 32 = 2 N +52).
11. Sample-standard Phase: Pull the TC, NSB, BC, and STD tubes and set aside. Into all sample tubes, pipette 150 µL of IGF-1 Assay buffer (PBSG:PBS-EDTA 1:100). Then into appropriate duplicate tubes, pipette 50 µL of the sample. Pipette directly down into the buffer.
12. Pipette 200 µL of IGF-1 Assay Buffer into all NSB and BC tubes. For the STD, first pipette the appropriate amount of IGF-1 assay buffer to make the difference between the 8 standard volumes and 200 µL (that is 198, 196, 192, 185, 175, 150, 100, and 0 µL), Then pipette the appropriate amount of standard (50ng/mL) into the tubes (2, 4,8,15, 25, 50, 100, 200 µL, respectively). Pipette standard directly into the buffer.
13. Antibody Phase: Put all tubes except TC and NSB back into the racks in numerical order. To all other tubes, add 200 µL of IGF-1 Antiserum (1:60,000). To the NSB tubes, add 200 µL of IGF-1 NSB solution (PBSG:PBS-EDTA:protamine sulfate 100mL:100mL:0.2mg/mL).
14. Place the NSB and TC tubes in racks an vortex all. Cover, label, and incubate 24-48 hr in fridge (48 is ideal but 24 is okay if in a hurry).

Day 4 (or 3) – Hot Hormone Addition:
15. Remove all racks from fridge. Add 200 µL of 125I-IGF-1 solution to all tubes (this solution should be between 40,000 and 60,000cpm/200 µL).
16. Cover and vortex. Incubate in fridge 24-48 hours (48 is ideal…).

**Day 6 (or 5 or 4) – Second Antibody Precipitation:**

17. Remove racks from fridge. To all tubes except TC, add 200 µL of 4X NRS (1:105). Follow this with 200 µL of 4X pARGG (1:4.5). Vortex all tubes and incubate in fridge over night (24 hrs is ideal; however precipitation likely is complete in 12-18 hrs.)

**Day 7 – pour off:**

18. Load all tubes except TC into centrifuge carriers. Spin at 3,000 rpm for 30 minutes. Immediately when centrifuge stops, remove, decant and drain and blot on paper towels. Add 1 mL cold PBS to all tubes, and repeat centrifuge-decant-drain-blot.
19. Count all tubes and save on disk. Adjust raw cpm’s in DETECTOR.XLS. Save adjusted Data. Copy into current RIANAL.123. The following standard doses should be used 62,124,248, 465, 775, 1550, 3100, 6200 ng
VITA

Patricia Terrell Richardel was born on August 18, 1979, in Metairie, Louisiana, to Gordon and Pat Terrell. After graduating from Mount Carmel Academy in 1997, she began her undergraduate studies in animal science at Louisiana State University. In December, 2001, she received her Bachelor of Science degree in Animal Science. After taking nine months off, she returned to Louisiana State University as a graduate student in the Department of Dairy Science in the area of dairy calf nutritional physiology. She will receive the degree of Master of Science in August of 2004. Upon completion, she will join the class of 2008 at the Louisiana State University School of Veterinary Medicine to strive toward a doctoral degree in veterinary medicine.