2007

Effects of protein sources on growth and hormonal status of weaned dairy calves

Christopher Aaron Sissell
Louisiana State University and Agricultural and Mechanical College

Follow this and additional works at: https://digitalcommons.lsu.edu/gradschool_theses

Part of the Animal Sciences Commons

Recommended Citation
https://digitalcommons.lsu.edu/gradschool_theses/435

This Thesis is brought to you for free and open access by the Graduate School at LSU Digital Commons. It has been accepted for inclusion in LSU Master's Theses by an authorized graduate school editor of LSU Digital Commons. For more information, please contact gradetd@lsu.edu.
EFFECTS OF PROTEIN SOURCES 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, Dairy and Poultry Sciences

by

Christopher Aaron Sissell
B.S., Louisiana State University, 2005
August, 2007
ACKNOWLEDGEMENTS

Graduate school has been both a difficult and exciting experience for me and there are many people who helped me along the way. I would first like to thank Dr. Cathy Williams for giving me the opportunity to pursue a Master’s degree. I would also like to thank my committee members, Dr. Bruce Jenny, Dr. Charles Hutchison, and Dr. Vinicius Moreira, for being patient with me to get my thesis finished.

Thank you to all the student workers who helped me out at the farm and in the lab. I especially want to thank Loretta Morante, Caitlin Contreary and Ashley Howard… for helping me with the hundreds of kjeldahls and numerous amounts of assays that had to be done. I am greatly appreciative to Mr. Danny Gantt, who helped me out at the farm during my experiment and for keeping his cool when dealing with the number of blown catheters that occurred. He was always there to help with the calves when I needed him and looked after them when I couldn’t.

I would like to thank Tony Bridges for giving up his time to help me feed my steers, clean pens, and assist with the 6 hour bleeds. He was a tremendous help both on the farm and in the classroom, without him, graduate school wouldn’t have been as much fun. I would also like to express my gratitude to my major professor Dr. Cathy Williams for her guidance, knowledge and patience. She kept me in line and always pushed me to do my best work. Without these two people I would have never made it this far.

I would also like to thank Randy Morrell for his help on the farm, which made my project possible. He has taught me many things about the dairy industry and a lot of the knowledge I learned was due to the hands-on experience that he provided.
Last but not least, I would like to thank my parents, Kim and Ray Sissell, who supported me throughout college and graduate school. They were always behind me in everything I did and I wouldn’t be the person I am today if it wasn’t for their love and support.
# TABLE OF CONTENTS

ACKNOWLEDGEMENTS ........................................................................................................ ii

LIST OF TABLES .................................................................................................................. vi

LIST OF FIGURES .............................................................................................................. vii

ABSTRACT ........................................................................................................................ viiii

CHAPTER

1. INTRODUCTION ........................................................................................................... 1

2. REVIEW OF LITERATURE ....................................................................................... 4
   Background ...................................................................................................................... 4
   Rumen Undegradable Protein ..................................................................................... 5
   Animal Protein By-Products ....................................................................................... 7
   A Description of the Protein Ingredients Used in this Study ....................................... 7
   Development of RUP Sources .................................................................................... 9
   Anabolic Hormones .................................................................................................... 10
   Effect of Post Ruminal Supply of Amino Acids on Hormonal Status ....................... 11

3. MATERIALS AND METHODS .................................................................................. 13
   Animals and Dietary Treatments .............................................................................. 13
   Sample Collection ...................................................................................................... 16
   Laboratory Methods .................................................................................................. 17
   Statistical Analysis .................................................................................................... 17

4. RESULTS AND DISCUSSION ................................................................................. 19
   Performance Data ...................................................................................................... 19
   Nitrogen Balance and Hormone Data ....................................................................... 22

5. SUMMARY AND CONCLUSION ............................................................................. 32
   Summary ...................................................................................................................... 32
   Conclusion .................................................................................................................. 33

REFERENCES .................................................................................................................... 34

APPENDIX A. PLASMA INSULIN RADIOIMMUNOASSAY ........................................... 41

APPENDIX B. PLASMA UREA NITROGEN COLORIMETRIC ASSAY .............................. 43

APPENDIX C. PLASMA GROWTH HORMONE RADIOIMMUNOASSAY ....................... 44
APPENDIX D. PLASMA INSULIN-LIKE GROWTH HORMONE RADIOIMMUNOASSAY ................................................................................45

VITA .................................................................................................................47
LIST OF TABLES

1. Composition of experimental diets.................................................................14

2. Least squares means for chemical analysis of the experimental diets.................15

3. Least squares means of average dry matter intake, body weight gain, and skeletal growth in steers fed experimental diets containing either SBM, Extruded-Expelled SBM, SoyPLUS®, or PRO-LAK..........................................................................................................................20

4. Least squares means of digestibility of dietary components of experimental diets containing either SBM, Extruded-Expelled SBM, SoyPLUS®, or PRO-LAK fed to steers..........................................................................................................................23

5. Least squares means for nitrogen balance in Holstein steers fed experimental diets containing either SBM, Extruded-Expelled SBM, SoyPLUS®, or PRO-LAK..........................................................................................................................24

6. Least squares means of plasma concentrations of insulin, growth hormone, and insulin-like growth factor-1 for steers fed experimental diets containing either SBM, Extruded-Expelled SBM, SoyPLUS®, or PRO-LAK..........................................................................................................................26
LIST OF FIGURES

1. Plasma insulin concentrations during a 6 hour period in Holstein steers fed diets containing SBM, Extruded-Expelled SBM, SoyPLUS®, or PRO-LAK...........................................................................................................................27

2. Plasma growth hormone concentrations during a 6 hour period in Holstein steers fed diets containing SBM, Extruded-Expelled SBM, SoyPLUS®, or PRO-LAK..........................................................................................................................30

3. Plasma insulin-like growth factor-1 concentrations during a 6 hour period in Holstein steers fed diets containing SBM, Extruded-Expelled SBM, SoyPLUS®, or PRO-LAK..............................................................................................................................31
ABSTRACT

Eight Holstein calves approximately 6 months of age (mean BW 185.15 ± 16.16 kg) were used in a replicated 4 x 4 Latin Square experiment to study effects of protein sources on performance of weaned dairy calves. Dietary treatments consisted of 16% CP diets with three sources of ruminally undegradable protein (RUP). Experimental diets were corn-silage based, with soybean meal (SBM) as the source of ruminally degradable protein (control) and 3 sources of RUP including heat treated soybean meal (SBM) (SoyPLUS®), animal protein blend (PRO-LAK), and extruded-expelled SBM, included at 45% of the dietary CP. The animals were fed their respective diets twice daily ad libitum. Animals were housed in individual stalls for 14 days for dietary adjustment and feed intake measurements. Steers were housed in metabolism crates during the last 4 days of each experimental period for sample collection. Total fecal and urine output was collected, weighed, and sampled for laboratory analysis of nitrogen during the 4-d collection period. On day 4 of the collection period, animals were fitted with jugular catheters. Blood samples were collected at 15-minute intervals for 6 hours for analysis of growth hormone. An additional blood sample was collected at time 0 for plasma urea nitrogen (PUN) and at 30-minute intervals for IGF-I and insulin. On day 18 of each experimental period body weight, wither height, hip height, and body length were measured. Treatment did not affect dry matter intake (P > 0.05). There were also no effects (P > 0.05) of protein source on nitrogen metabolism, PUN, or growth parameters. There was no effect (P > 0.05) of protein source on GH levels. There was no effect of RUP sources on plasma IGF-I concentrations on (P > 0.05). Treatment did not affect (P > 0.05) insulin concentrations. However, there was an affect of time on insulin concentrations (P < 0.05). There was no difference (P >0.05) among treatments in the
digestibility of DM, OM, NDF, ADF, or CP. These data suggest that feeding diets with sources of RUP does not improve performance in weaned dairy calves.
CHAPTER 1

INTRODUCTION

The goal of replacement heifer programs is to provide the opportunity for the heifer to fully develop her lactation potential at the desired age with minimal expense. There is considerable interest in rapid growth rates for replacement heifers because breeding date and subsequent age at calving is determined by size. Replacement heifers represent a sizable investment and are considered the future of the dairy herd. Unfortunately on many farms, the dairy heifer is the most over looked and undermanaged asset on the farm (Tozer et al., 2007). Therefore, proper nutrition, along with management, is necessary to produce large, healthy heifers that can be bred at an early age. Inadequate size at first calving may limit milk production and conception rates during the first lactation. The goal should be to grow heifers rapidly enough to freshen at least 24 months of age with minimal expense. Delaying calving can add considerable costs for each month beyond 24 months of age, mainly due to feed costs. According to Gill and Allaire (1976) heifers should calve between 22.5 and 23.5 months of age to maximize total lifetime performance. Keown and Everett (1986) found that first lactation milk production was optimized with a postcalving body weight of 570 kg. To weigh 570 kg after calving at 24 months of age, heifers need to gain on average a minimum of 820 g/d from birth to calving (Keown and Everett, 1986). Therefore, it is critical that dairy farmers minimize the costs of raising heifers by calving them at younger ages and maximizing growth rates without excessive conditioning (Lammers et al., 1999). With increasing emphasis on accelerated growth rates and earlier age at first calving, nutritional management of replacement heifers is extremely important (Richardel, 2004).
In the past, overfeeding of protein sources supplied enough excess nitrogen (N) maximal microbial growth while ensuring some protein escaped rumen degradation and was absorbed by the intestine to supply the balance of the animal’s protein requirement (Jayawardena, 2000). However, protein is too expensive to waste. More recently, dairy nutritionists began to identify protein sources that partially escape rumen degradation and increase the supply of metabolizable amino acids (AA) to the animal (Jayawardena, 2000). It has become evident that the quality of the rumen-escape protein is important and quality of protein is expressed both in terms of its intestinal digestibility as well as its amino acid profile (Santos et al., 1998; Calsamiglia and Stern, 1995). The metabolizable protein requirement of low-producing ruminants can be met by microbial protein; however, as production increases (early rapid growth, high wool and/or milk production), it becomes more important to provide rumen undegradable protein (RUP). Supplementing ruminant diets with RUP can increase the flow of nitrogen (N) and amino acids to the small intestine resulting in improved growth and efficiency of N utilization (Bohnert et al., 2007).

Growth is a complex, highly integrated process involving numerous interactions among nutrients, environment, genotype, and many different hormones and receptors of these hormones in various tissues (Spencer, 1985). Nutrition is an important factor that affects the rate and extent of growth along with anabolic hormones. Barry et al. (1982) suggested that changes in hormonal status from increased or altered balance of AA absorbed could increase BW and protein gain. While many hormones influence growth, the primary regulator of postnatal growth is somatotropin, otherwise known as growth hormone (GH). Many of the anabolic effects of GH however are modified by insulin (INS), and mediated by insulin-like growth factor-1 (IGF-1) (Guyton and Hall, 2000). Nutritional modification may positively affect growth through
increased production of anabolic hormones. This could be advantageous in the pre-pubertal heifer by improving growth rates and skeletal development.

Previous research has shown that postruminal supplies of AA increased N retention and could support increased rates of gain (Richardson and Hatfield, 1978). Post ruminal supply of AA via abomasal infusion has also resulted in increase INS, GH, and IGF-1 (Ragland-Gray et al., 1997; Davenport et al., 1995b; Guerino et al., 1991; Davenport et al., 1990b) in growing ruminants. If greater AA flow were provided post ruminally from dietary sources circulating GH concentrations could be elevated, perhaps along with IGF-1, which mediates the effects of GH (Gluckman et al., 1987). Therefore, the objective of this study was to determine the effects of rumen undegradable protein sources on nitrogen balance, anabolic hormonal status, and growth in weaned dairy calves.
CHAPTER 2
REVIEW OF LITERATURE

Background

In growing calves, microbial protein is unable to meet metabolizable amino acid (AA) requirements, and therefore high-quality ruminally undegradable protein (RUP) has to be provided (NRC, 1996). Therefore, there has been interest in formulating diets and(or) supplements for ruminants to provide a supply or assortment of AA to the small intestine (SI). The dietary deficiency provided by microbial protein can be corrected by feeding supplemental protein sources (Richardel, 2004.) Supplementation with RUP may supply limiting amino acids directly to the small intestine. However, RUP protein sources vary in their amino acid composition (Merchen and Titgemeyer, 1992), and may increase the supply of metabolizable protein, but not alleviate the supply of specific amino acids. For example, meat and bone meal is a good source of RUP, but it is deficient in methionine which is a limiting AA for growth in ruminants (Klemesrude et al., 2000). Blood meal, also an excellent source of RUP, may be deficient in sulfur amino acids; however the addition of feather meal improved daily gain and protein efficiency in growing steers, compared with either meal fed alone (Goedeken et al., 1990). It is important to match the protein supplement with the needs of the animal in order elicit a growth response.

With great concerns today about the environment and the amount of nitrogen (N) excreted, heifers and steers 6 months to breeding age do not need increased levels of crude protein above 16%. Richardel (2004) found that diets greater than 16% crude protein did not improve performance in weaned dairy calves, therefore, supplementing protein in the form of
RUP in the diet maybe a better alternative. Increasing RUP could help increase the amount of AA reaching the small intestine while limiting N excretion in the environment

**Rumen Undegradable Protein**

Microbial protein is an excellent quality protein; unfortunately not enough can be produced to supply the requirements for growing ruminants. Therefore, undegradable protein must be available to make up the difference between what the heifer requires and what the microbial protein supplies. Rumen undegradable protein is the second most important source of absorbable AA to the animal, with microbial protein being the first. These types of protein resist degradation by ruminal microbes and reach the abomasum and small intestine essentially unaltered (Merchen and Titgemeyer, 1992). Feeds vary in their ability to supply RUP and quality will vary by source and depend on digestibility and amino acid composition (Stallings, 2001). The AA profile available for absorption is determined predominantly by the AA profile of the RUP supplement. If the RUP is indigestible or has a poor amino AA composition it will provide little value to the animal.

Research has shown that high RUP diets (40 to 50% of CP) can improve growth and feed efficiency of dairy heifers and steers (Bethard et al., 1997). Thonney and Hogue (1986) reported improved feed efficiency, but there was no improvement in average daily gains (ADG), when cottonseed meal was replaced with fishmeal in the diet of growing Holstein steers. Similar results were reported by Bethard et al. (1997) where 6 to 13 month old heifers who received a high level of RUP in the diet (blood meal) were more efficient per unit of dry matter (DM) and total digestible nutrients (TDN) than those heifers who consumed low RUP level in the diets. Improved feed efficiency was reported when RUP increased from 31% to 55% of CP, using blood meal as a source of RUP (Tomlinson et al., 1997). Swartz et al. (1991) reported that high
RUP diets (37.9 to 46.4% of CP) supplemented with blood meal resulted in improved feed efficiency from 14 to 25 wk of age but not from 1 to 13 wk of age when compared with a low RUP (29.7 to 32.9% of CP) diet containing soybean meal.

Supplementing ruminant diets with ruminally undegradable proteins can increase the flow of N and AA to the small intestine (Titgemeyer et al., 1989; Zinn et al., 1981) and result in improved growth and efficiency of N utilization (Goedeken et al., 1990; Stock et al., 1981). MacRae and Ulyatt (1974) showed increased AA absorption was positively correlated with weight gain. In an experiment feeding fresh cut tall fescue, performance of lambs was increased by supplementation of energy or energy and RUP (Daura and Reid, 1991). Furthermore, lambs fed the high RUP levels gained faster and retained more nitrogen than lambs supplemented with energy only (Daura and Reid, 1991). Similar results were found when Veira et al. (1988) fed ad libitum grass silage, supplemented with fish meal, to beef steers. The steers had increased ADG compared to those supplemented with barley only. From those results it was determined that the improved protein to energy ratio enhanced the animal’s ability to deposit protein and therefore increase the growth of the beef steers. Alkire et al. (2007) found that crossbred steers and heifers fed a treatment that consisted of corn or citrus pulp with added RUP (SoyPLUS®) at 5 different levels, 0.0, 0.055, 0.11, 0.165, and 0.22 kg/d, had increased ADG. The ADG increased linearly as the level of RUP was increased, and ADG for the highest amount of RUP fed improved by 0.393 kg over the control. Zerbini and Polan (1985) observed improved ADG, but no differences in DMI, when fishmeal was used as a source of RUP in diets of Holstein bull calves.

While many of these researchers found improved performances of ruminants fed RUP, many have reported no responses to feeding RUP, decreases in total N flow to the small intestine, and/or decreased microbial protein synthesis (Loerch and Berger, 1983; Plegge et al.,
There have been many reasons as to why the animals are not responding to the inclusion of RUP in the diet. One reason is the actual RUP level of protein sources deviated from published values (Erasmus et al., 1988; Titgemeyer et al., 1989; Lardy et al., 1993), which is one of the plausible reasons for the lack of consistent responses (Legleiter et al., 2005). Also, too much ruminally undegradable protein can decrease the efficiency of microbial protein synthesis and flow of microbial AA to the small intestine (Siddons et al., 1985; Cecava et al., 1991) compared with feeding more degradable protein sources (Cecava et al., 1993), which defeats the purpose of feeding RUP in the diet. Another reason may be lower concentrations of end products from ruminal proteolysis, such as NH3 N, AA, or branched chain VFA, may limit microbial protein synthesis when diets contain high levels of ruminally undegradable protein (Cecava et al., 1993).

**Animal Protein By-Products**

Animal protein sources are high in protein content and are generally much more resistant to microbial degradation in the rumen than most of the commonly fed plant protein sources. In general, ruminant protein degradation of animal proteins rank, from bottom to top, is: blood meal; hydrolyzed feather meal; meat and bone meal; fish meal (Staples, 1992). Caution is suggested in using animal protein feedstuffs because palatability, cost per unit of protein, consistency of product and impact on animal performance are key factors to successful dairying (Staples 1992).

**A Description of the Protein Ingredients Used in this Study**

**Soybean Meal** This byproduct feed is known to be the most popularly used protein ingredient in dairy cattle diets. Soybean meal is produced during the extraction of oil from
soybeans by solvent or mechanical methods, and then is toasted and ground during processing (Jayawardena, 2000). The protein content of soybean meal ranges from around 44 to 48% CP and around 35% of SBM is rumen undegradable. Soybean meal contains lysine which is a limiting amino acid in dairy cattle. The popularity of soybean meals in dairy cattle diet formulation is attributed to several factors including widespread availability, palatability, and high protein and energy contents (Jayawardena, 2000).

**PRO-LAK** PRO-LAK is a specially formulated, marine and animal bypass protein supplement designed for cattle requiring additional essential amino acids. PRO-LAK is a fish meal based product. It also contains high quality blood meal, hydrolyzed feather meal, meat and bone meal, and synthetic methionine blended to supply the 10 essential amino acids. PRO-LAK is formulated for 65% of the crude protein to bypass the rumen (Baker & Bro, 2007).

**SoyPLUS®** SoyPLUS® is an expeller-cooked soybean meal. SoyPLUS® offers at least 60% bypass protein and at least 95% digestibility. The SoyPLUS® modified expeller extraction system is based primarily on the normal expeller extraction method. However, the SoyPLUS® method contains additional reaction and conditioning steps, which are designed to create high levels of bypass protein without damaging the protein quality. The SoyPLUS® method is so unique that it has been granted a patent (U.S. Patent #5,225,230) (West Central, 2007).

**Extruded-Expelled SBM** Extruded-expelled SBM is produced by mechanical friction creating a high temperature for a short time period which destroys the tripsin-inhibiting enzymes. There are several extruder-expeller processed SBM products available to purchase. Company testing for the ruminal undegradability of the protein in these products is extremely limited. Company nutrient profile sheets generally indicate a higher fat content than reported for cooker-expeller products (Shaver, 2007).
Development of RUP Sources

Heat processing decreases rumen protein digestion by denaturation of proteins and by the formation of protein-carbohydrate and protein–protein cross-links (NRC, 2001). Broderick and Craig (1980) concluded that the heat treatment decreases ruminal degradation partly by blocking reactive sites for microbial proteolytic enzymes and partly by reducing protein solubility. Some commercial methods that rely solely on heat include cooker-expeller processing of oilseeds, additional heat treatment of solvent extracted oilseed meals, roasting, extrusion, and pressure roasting. Heat processing must be under careful control in order to optimize the content of digestible RUP (Schwab, 1995). Heat treatment protects dietary proteins for ruminants, but it is important that appropriate temperature and heating times are employed for particular feeds (Kempton et al., 1978). Under-heating can result in only a small increase in RUP. Over-heating can reduce the intestinal digestibility of RUP through the formation of indigestible Maillard products and protein complexes (Van Soest, 1994). The content of sugars influences the extent of heat “damage” (Kempton et al., 1978). Over-heating also causes absolute losses of lysine, cystine, and arginine. Lysine is the most sensitive to heat damage and undergoes both destruction and decreased availability (NRC 2001). When done properly, heat processing decreases ruminal protein degradability without having a significant decrease in AA losses.

Chemical treatment of feed proteins can be divided into three categories: (1) chemicals that combine with and introduce cross-links in proteins (e.g., aldehydes), (2) chemicals that alter protein structure by denaturation (e.g., acids, alkalis, and ethanol), and (3) chemicals that bind to proteins but with little or no alteration of protein structure (e.g., tannins) (NRC, 2001). The use of chemicals as the sole treatment for increasing the RUP content of feed proteins has not received commercial acceptance (NRC, 2001). Since chemical treatment alone hasn’t been
accepted, a more effective approach has been taken that involves a combination of chemical and heat treatment.

**Anabolic Hormones**

Insulin is synthesized in the pancreas within the β-cells of the Islets of Langerhans. It is a polypeptide hormone that regulates carbohydrate metabolism. Apart from being the primary agent in carbohydrate homeostasis, it has effects on fat metabolism and changes the liver's activity in storing or releasing glucose. Insulin also promotes protein synthesis and thus has a permissive effect on growth hormone (GH) (Hadley, 2000; Guyton and Hall, 2000). Insulin increases DNA replication and protein synthesis via control of AA uptake (Hadley and Levine, 2006).

Growth hormone is a 191-amino acid, single chain polypeptide hormone which is synthesized, stored and secreted by the somatotrope cells within the lateral wings of the anterior pituitary gland, and stimulates growth and cell reproduction in humans and other animals. Like most other protein hormones GH acts by interacting with a specific receptor on the surface of cells. Secretion levels of GH are highest during puberty. 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). Effects of growth hormone on the tissues of the body can generally be described as anabolic (building up). Growth hormone increases calcium retention, strengthens and increases the mineralization of bone, increases muscle mass through creation of new muscle cells, promotes lipolysis, which results in the reduction of adipose tissue (body fat), increases protein synthesis and stimulates the growth of all internal organs excluding the brain. GH also
stimulates production of insulin-like growth factor-1 (IGF-1). The liver is a major target organ of GH for this process, and is the principal site of IGF-1 production (Hadley and Levine, 2006).

Insulin-like growth factor-1 is a polypeptide protein hormone that consists of 70 amino acids in a single chain. It plays an important role in growth and continues to have anabolic effects in adults. IGF-1 is mainly secreted by the liver as a result of stimulation by GH. Because of its strong affinity for its plasma binding proteins, IGF-1 prolongs the growth promoting effects of GH (Hadley, 2000). Almost every cell in the human body is affected by IGF-1, especially cells in muscle, cartilage, bone, liver, kidney, nerves, skin, and lungs. In addition to the insulin-like effects, IGF-1 can regulate cell growth and development, especially in nerve cells, as well as cellular DNA synthesis (Hadley and Levine, 2006).

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

The rate and extent of growth is impacted not only by nutrition but hormonal factors with the majority of control being through the actions of the endocrine system. It is thought that by increasing the flow of AA to the abomasum from RUP could stimulate secretion of anabolic hormones and therefore influence growth (Richardel, 2004). Barry et al. (1982) suggested that changes in hormonal status from increased or altered balance of AA absorbed could increase BW and protein gain. If greater AA flow or a specific increase in arginine of dietary origin were provided post-ruminally, circulating GH concentrations could be elevated, perhaps along with IGF-I, which mediates the effects of GH (Gluckman et al., 1987). Greater AA absorption from the small intestine could stimulate anabolism by increasing insulin secretion (Davis, 1972).

Davenport et al. (1995b) studied the relationship between dietary N and IGF-1 in lambs. He found that increasing dietary N intake correlated with high levels of serum IGF-1. The data
suggested that a relationship between IGF-1 and N status of growing ruminants may be dependent on post-ruminal availability of AA. A post-ruminal supply of AA, supplied by AA bypassing degradation in the rumen, affected hormonal status of growing Holstein steers abomasally infused with casein and arginine (Ragland-Gray et al., 1997). They reported an increase in GH concentrations when arginine was infused along with an increase in growth and INS and IGF-1 concentrations when casein was infused. Davenport et al. (1995b) reported abomasal arginine infusions resulted in increased GH concentrations when compared to the control; whereas, the infusions of casein resulted in an increase in serum levels of IGF-1 and INS. Supplementing the diet of growing heifers with RUP may provide enough AA to the small intestine to improve growth and development by influencing the anabolic hormones.
MATERIALS AND METHODS

Animals and Dietary Treatments

Eight weaned Holstein steers approximately 6 months of age (mean BW 185.15 ± 16.16 kg) were utilized in a 12-wk experiment to determine the effects of three different RUP sources on growth and anabolic hormone status. The eight steers utilized in this study were born on the LSU Agricultural Center Dairy Research and Teaching Farm in Baton Rouge, LA and were 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 SoyPLUS® (West Central, Ralston, IA), PRO-LAK (H.J. Baker & Bro., Inc., Westport, CT), or Extruded-Expelled Soybean Meal (Ferriday Oil Seed Pro, LLC, Ferriday, LA) as the sources of RUP. The treatments consisted of diets formulated to contain 1) 16% CP with SBM 2) 16% CP with Extruded-Expelled SBM  3) 16% CP with SoyPLUS® 4) 16% CP with PRO-LAK. Compositions of the experimental diets are listed in Table 1. Chemical analyses of the diets are listed in Table 2. Four individual grain mixes were made that contained SBM, Extruded expelled SBM, SoyPLUS® or PRO-LAK. Each of the diets was formulated to contain 45% of the CP as RUP. The individual diets were mixed daily with a portable cement mixer and fed as a total mixed ration. The animals were fed their respective diets twice per day at 8 a.m. and 4:30 p.m. ad libitum during each 18-d experimental period, which included a 14-d adjustment period and a 4-d sample collection period. Water was available ad libitum. Animals were housed in individual pens for 14 d during the adjustment period and in metabolism crates (152.4 cm x 76.2 cm x 121.92 cm) for 3 d during each period.
Table 1. Composition of the experimental diets.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Soybean meal</th>
<th>Extruded-Expelled SBM</th>
<th>SoyPLUS®</th>
<th>PRO-LAK</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of DM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corn Silage</td>
<td>32.17</td>
<td>32.17</td>
<td>32.17</td>
<td>32.17</td>
</tr>
<tr>
<td>Whole Cotton seed</td>
<td>3.49</td>
<td>0.00</td>
<td>3.49</td>
<td>6.30</td>
</tr>
<tr>
<td>Soybean Hulls</td>
<td>20.71</td>
<td>16.24</td>
<td>18.97</td>
<td>18.44</td>
</tr>
<tr>
<td>Ground Corn</td>
<td>13.35</td>
<td>13.35</td>
<td>14.72</td>
<td>17.37</td>
</tr>
<tr>
<td>Cottonseed Hulls</td>
<td>15.63</td>
<td>18.36</td>
<td>14.26</td>
<td>14.49</td>
</tr>
<tr>
<td>Mineral¹</td>
<td>1.90</td>
<td>1.90</td>
<td>1.90</td>
<td>1.90</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>12.82</td>
<td>0.00</td>
<td>1.37</td>
<td>3.64</td>
</tr>
<tr>
<td>Extruded-expelled SBM</td>
<td>0.00</td>
<td>17.98</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>SoyPLUS®</td>
<td>0.00</td>
<td>0.00</td>
<td>13.20</td>
<td>0.00</td>
</tr>
<tr>
<td>PRO-LAK</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>5.69</td>
</tr>
</tbody>
</table>

¹Custom Heifer Mineral Mix: 18.30% Ca, 2.13% P, 3.70% Mg, 1.5% K, 9.92% Na, 15.49% Cl, 0.69% S, 23 ppm Co, 594 ppm Cu, 4212 ppm Fe, 67 ppm I, 1967 ppm Mn, 18 ppm Se, 2199 ppm Zn, 184 KIU/lb Vitamin A, 67 KIU/lb Vitamin D, 1056 IU/lb Vitamin E, 816 mg/lb Bovatec™.
Table 2. Least squares means for the chemical analysis of experimental diets.

<table>
<thead>
<tr>
<th>Item</th>
<th>Soybean Meal</th>
<th>Extruded-expelled SBM</th>
<th>SoyPLUS</th>
<th>PRO-LAK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter, %</td>
<td>51.81</td>
<td>51.61</td>
<td>51.12</td>
<td>51.97</td>
</tr>
<tr>
<td>% of DM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Organic Matter</td>
<td>90.14</td>
<td>89.27</td>
<td>89.98</td>
<td>89.19</td>
</tr>
<tr>
<td>CP(^1)</td>
<td>16.12</td>
<td>16.67</td>
<td>15.57</td>
<td>15.97</td>
</tr>
<tr>
<td>ADIN(^2)</td>
<td>0.34</td>
<td>0.35</td>
<td>0.36</td>
<td>0.36</td>
</tr>
<tr>
<td>NDF(^3)</td>
<td>50.89</td>
<td>46.81</td>
<td>50.80</td>
<td>49.81</td>
</tr>
<tr>
<td>ADF(^4)</td>
<td>32.50(^A)</td>
<td>29.82(^C)</td>
<td>30.28(^{BC})</td>
<td>31.13(^B)</td>
</tr>
</tbody>
</table>

\(^1\)CP = N x 6.25.  
\(^2\)ADIN = acid detergent insoluble nitrogen  
\(^3\)CP = N x 6.25.  
\(^4\)ADIN = acid detergent insoluble nitrogen
Sample Collection

At the beginning of each experimental period and then on d 18 of each experimental period body weight, wither height, hip length, and body length were measured. On d 14 to 18 total fecal output was collected, weighed, and a subsample was reserved for laboratory analysis. Subsamples were dried to a constant weight at 55°C, combined 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 14-18 where volume and weight was measured. Urine was preserved between collections in 6 N HCl (100 mL). As the steers grew, 200 mL of HCL was needed to preserve the large amounts of urine that was being collected by certain steers. Urine was combined by steer and period and frozen until analyzed for N.

Diet and ort grab samples from the individual steers were collected every other day during the 14-day adjustment period and diets were collected daily during the 3 day collection 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, and subsampled and analyzed for ash, DM, ADF, NDF and N.

On d 18 of each experimental period, animals were fitted with 14G x 5.1cm indwelling jugular catheters (Baxter Healthcare Corporation, Deerfield, IL). A 6% sodium citrate solution was used to flush catheters before and between sampling. A blood sample was collected for each steer for plasma urea nitrogen after catherization. Blood samples were obtained from each steer at 15-min intervals for 6 h for analysis of GH, with additional blood samples from each steer
collected at 30-min intervals for analysis of IGF-1 and INS. Feed and water were available to
the steers during the 6 hour blood sample collection period. 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 -20°C 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 was analyzed for urea
nitrogen concentrations using commercial spectrophotometric kits. 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 a
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 Analysis**

Data for growth, intake, digestibility, diet composition, N balance, and PUN 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 period and treatment. Square, steer, and all
appropriate interactions involving these terms were included as random effects. Data for IGF-1,
INS, and GH were analyzed as repeated measures nested within the Latin square using a mixed model (Littell et al., 1998). The model included terms for the fixed effects of period and treatment. Square, period, steer, and all appropriate interactions were included as random effects. Time was included as a repeated term and was modeled with an autoregressive covariance structure (AR (1)). All data are presented as least squares means. All calculations were completed using SAS (SAS Institute Inc., Cary, NC, 1990). Statistical significance was declared at probabilities < 0.05.
CHAPTER 4

RESULTS AND DISCUSSION

**Performance Data**

Least square means for average daily DMI and daily increases in growth parameters are listed in Table 3. There was no effect (P > 0.05) of RUP on mean daily DMI. Legleiter et al. (2004) also reported no difference in DMI among treatments when crossbred steers were fed increasing levels of arginine, 0.5, 1, 1.5, and 2x the required levels of arginine, with spray-dried blood meal as the source of protein. Their daily DMI averaged 7.6 kg/steer and did not differ among treatments. Coomer et al. (1993) also found no effect of RUP in the form of heat-treated SBM, corn gluten meal or a combination of both, on DMI when a TMR diet containing sorghum silage was fed to Holstein steers. Davenport et al. (1990a) reported no difference in DMI in crossbred beef calves feed *ad libitum* corn silage supplemented with ground SBM with or without FM and or rumen protected lysine. Zerbini and Polan (1985) found improved ADG, but no differences in DMI, when fish meal replaced soybean meal in diets of 9-wk-old Holstein bull calves.

There was no effect (P > 0.05) of RUP source on ADG, or increases in wither height, hip height, or body length (Table 3). Devant et al. (2000) fed twenty crossbred heifers two protein sources differing in ruminal degradability (58% vs 42% of CP for SBM and treated SBM, respectively) and observed no improvements in ADG or DM intake. 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 blood meal. Coomer et al. (1993) also found no effect of RUP
Table 3. Least squares means of average daily dry matter intake, body weight gain, and skeletal growth in steers fed diets containing either SBM, Extruded-Expelled SBM, SoyPLUS or PRO-LAK.

<table>
<thead>
<tr>
<th></th>
<th>Soybean meal</th>
<th>Extruded-Expelled meal</th>
<th>SoyPLUS</th>
<th>PRO-LAK</th>
<th>SEM</th>
<th>P=</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMI (kg/d)</td>
<td>7.39</td>
<td>7.41</td>
<td>7.25</td>
<td>7.02</td>
<td>0.26</td>
<td>0.3728</td>
</tr>
<tr>
<td>ADG (kg/d)</td>
<td>1.22</td>
<td>1.17</td>
<td>1.23</td>
<td>1.15</td>
<td>0.19</td>
<td>0.9637</td>
</tr>
<tr>
<td>Feed Efficiency</td>
<td>6.97</td>
<td>6.57</td>
<td>6.28</td>
<td>6.32</td>
<td>0.93</td>
<td>0.7842</td>
</tr>
<tr>
<td>Wither height (cm/d)</td>
<td>0.27</td>
<td>0.17</td>
<td>0.22</td>
<td>0.12</td>
<td>0.05</td>
<td>0.2318</td>
</tr>
<tr>
<td>Hip height (cm/d)</td>
<td>0.16</td>
<td>0.20</td>
<td>0.24</td>
<td>0.20</td>
<td>0.03</td>
<td>0.4141</td>
</tr>
<tr>
<td>Body length (cm/d)</td>
<td>0.20</td>
<td>0.08</td>
<td>0.39</td>
<td>0.23</td>
<td>0.10</td>
<td>0.3162</td>
</tr>
</tbody>
</table>
supplementation on ADG when heat-treated SBM or corn gluten meal was fed to Holstein steers. However, Heinrichs (1996) fed a diet containing 18% CP and either 36% or 45% of CP as RUP. There were three treatments, a control with solvent extracted SBM, SoyPLUS®, or corn distillers and cottonseed meal combination. Body weights were significantly greater for calves fed SoyPLUS®. Also, calves fed SoyPLUS® outperformed the other calves in heart girth, height, and coccae width. Other studies have also shown that supplementation with RUP results in increased growth and ADG of beef and dairy calves (Dawson et al., 1991; Davenport et al., 1990a; Zerbini and Polan, 1985). While these studies have shown positive effects of increasing RUP Quigley and Bearden (1990) and Swartz et al. (1991) reported no change in daily gain as diets increased in RUP percentage. The lack of response when supplementing diets with RUP might have resulted from excess total protein, masking an effect of an undegradable protein source (Tomlinson et al., 1997).

There was no effect (P > 0.05) of RUP source on feed efficiency (Table 3). Other researchers found no response in ADG or feed efficiency with high RUP diets (Heinrichs et al., 1993; Mantysaari et al., 1989; Kertz et al, 1987). However, Tomlinson et al. (1997) reported improved feed efficiency as RUP increased from 31 to 55% of CP, using blood meal as a source or RUP. Similarly, Thonney and Hogue (1986) reported improved feed efficiency but not improved ADG when cottonseed meal was replaced with fish meal in the diet of growing Holstein steers. Swartz et al. (1991) reported that high RUP diets (37.9 to 46.4% of CP) supplemented with blood meal resulted in improved feed efficiency from 14 to 25 wk of age but not from 1 to 13 wk of age when compared with a low RUP (29.7 to 32.9% of CP) diet containing soybean meal.
Least squares means for digestibilities of dietary components are listed in Table 4. There was no difference (P > 0.05) among the experimental diets in the digestibility of DM, OM, NDF, ADF, or CP. Similarly, Devant et al. (2000) observed no effect on total tract apparent digestibility of DM or OM of twenty crossbred heifers fed dextrose treated SBM at 42% of CP. Coomer et al. (1993) reported that when steers were fed higher levels of RUP, flow of OM to the terminal ileum and fecal OM output increased; however, there was no difference in total tract OM apparent digestibility. These researchers also reported true OM digestion in the reticulorumen was reduced in steers receiving RUP compared with those receiving SBM. 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. Knaus et al. (1998) fed a dietary treatment that contained mixtures of RUP (meat and bone meal, blood meal, feather meal and fish meal) at 0, 2.5, 5 and 7.5% of the total mixed diet and the DM digestibility was not significantly affected by the varying amount of RUP addition.

**Nitrogen Balance and Hormone Data**

Nitrogen metabolism data are presented in Table 5. There was no effect (P > 0.05) of RUP source on nitrogen balance and PUN concentration of steers. Davenport et al. (1990a) reported no effect of treatment on PUN concentrations in crossbred beef calves fed ad libitum corn silage supplemented with SBM with or without FM and or rumen-protected lysine. Knaus et al. (2001) reported an increase in average daily N intakes for diets containing 2.6% and 5.2% RUP (meat and bone meal, fish meal, hydrolyzed feather meal, and blood meal), than the corn-based control diet. These
Table 4. Least squares means of digestibilities for experimental diets containing either SBM, Extruded-Expelled SBM, SoyPLUS® or PRO-LAK.

<table>
<thead>
<tr>
<th></th>
<th>Soybean meal</th>
<th>Extruded-Expelled SBM</th>
<th>SoyPLUS®</th>
<th>PRO-LAK</th>
<th>SEM</th>
<th>P=</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM, %</td>
<td>74.98</td>
<td>76.33</td>
<td>76.40</td>
<td>75.89</td>
<td>1.01</td>
<td>0.3291</td>
</tr>
<tr>
<td>OM, %</td>
<td>85.84</td>
<td>86.67</td>
<td>86.85</td>
<td>86.54</td>
<td>0.60</td>
<td>0.3161</td>
</tr>
<tr>
<td>CP, %</td>
<td>68.35</td>
<td>70.28</td>
<td>68.48</td>
<td>66.67</td>
<td>1.76</td>
<td>0.2472</td>
</tr>
<tr>
<td>NDF, %</td>
<td>64.17</td>
<td>64.58</td>
<td>67.19</td>
<td>66.69</td>
<td>2.15</td>
<td>0.5146</td>
</tr>
<tr>
<td>ADF, %</td>
<td>58.83</td>
<td>59.47</td>
<td>59.68</td>
<td>61.27</td>
<td>2.26</td>
<td>0.5450</td>
</tr>
</tbody>
</table>
Table 5. Least square means for nitrogen metabolism in Holstein steers fed experimental diets containing either SBM, Extruded-Expelled meal, SoyPLUS®, or PRO-LAK.

<table>
<thead>
<tr>
<th></th>
<th>Soybean meal</th>
<th>Extruded-Expelled meal</th>
<th>SoyPLUS®</th>
<th>PRO-LAK</th>
<th>SEM</th>
<th>P=</th>
</tr>
</thead>
<tbody>
<tr>
<td>N intake, g/d</td>
<td>177.95</td>
<td>181.69</td>
<td>172.06</td>
<td>167.93</td>
<td>10.73</td>
<td>0.7461</td>
</tr>
<tr>
<td>Fecal N, g/d</td>
<td>56.07</td>
<td>53.87</td>
<td>54.38</td>
<td>56.10</td>
<td>4.02</td>
<td>0.6812</td>
</tr>
<tr>
<td>Urinary N, g/d</td>
<td>83.26</td>
<td>90.18</td>
<td>72.80</td>
<td>83.91</td>
<td>21.71</td>
<td>0.7958</td>
</tr>
<tr>
<td>N balance, g/d</td>
<td>44.74</td>
<td>30.01</td>
<td>44.87</td>
<td>40.75</td>
<td>18.16</td>
<td>0.7850</td>
</tr>
<tr>
<td>N absorbed, g/d</td>
<td>121.88</td>
<td>127.82</td>
<td>117.68</td>
<td>111.83</td>
<td>8.23</td>
<td>0.5713</td>
</tr>
<tr>
<td>N digestibility, %</td>
<td>68.35</td>
<td>70.28</td>
<td>68.48</td>
<td>66.67</td>
<td>1.76</td>
<td>0.2472</td>
</tr>
<tr>
<td>PUN, mg/dL</td>
<td>8.54</td>
<td>7.98</td>
<td>7.46</td>
<td>7.39</td>
<td>1.11</td>
<td>0.5467</td>
</tr>
</tbody>
</table>
researchers also reported that fecal N excretion remained constant for all three RUP diets. The inclusion of 5.2% RUP in the diet resulted in a significant elevation of urinary N excretion compared with the control and 2.6% RUP diets. This contributed to a significantly lower daily N retention when comparing the 5.2% RUP and control diets, whereas N retention for the control and 2.6% RUP diets were not significantly different. Nitrogen retention was numerically higher in the 2.6% RUP diet (3.4 g/d, or 7%) than with the control diet, suggesting that the 2.6% RUP came closest to meeting MP and amino acid requirements (Knaus et al., 2001). However, Knaus et al. (1998) had an increase in apparent digestibility of N for 63.8 to 65.8, 70.7 and 71.5%, the amount of N absorbed increased from 73, 84, 100, and 106 g/d, and N balance increased from 20 to 30, 33, and 39 g/d when RUP was fed at 0, 2.6, 5.2 and 7.8% of diet DM, respectively. He also reported that circulating concentrations of PUN were increased from 4.5 for the control diet to 5.7, 6.2, and 6.1 mg/dL when the RUP mixture was added at 2.6, 5.2 and 7.8%, respectively. Knaus et al. (1998) and Robinson (1998) observed a significant improvement in apparent N digestibility when a combination of animal by-product protein sources was added to a corn-based diet fed to Holstein steers.

Least squares means for plasma concentrations of INS, GH, and IGF-1 are reported in Table 6. Insulin was not affected (P > 0.05) by any of the sources of RUP that were included in the diet. However, there was a main effect of time on INS concentration (P < 0.05) (Figure 1). Lalman et al. (1993) reported insulin concentrations were higher in heifers fed RUP (blood and corn gluten meal) at d 125, 130, and 140 than heifers fed a mixture of propionic acid and monensin. Increases in INS
Table 6. Least squares means for plasma concentrations of insulin, growth hormone, and insulin-like growth hormone for Holstein steers fed diets containing either SBM, Extruded-expelled meal, SoyPLUS® or PRO-LAK.

<table>
<thead>
<tr>
<th></th>
<th>Soybean meal</th>
<th>Extruded-expelled meal</th>
<th>SoyPLUS®</th>
<th>PRO-LAK</th>
<th>SEM</th>
<th>P=</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma Insulin (µIU/mL)</td>
<td>8.04</td>
<td>9.30</td>
<td>7.73</td>
<td>9.48</td>
<td>1.77</td>
<td>0.6668</td>
</tr>
<tr>
<td>Plasma Growth Hormone (ng/ml)</td>
<td>22.49</td>
<td>18.14</td>
<td>20.36</td>
<td>22.83</td>
<td>2.18</td>
<td>0.0658</td>
</tr>
<tr>
<td>Plasma IGF-1 (ng/mL)</td>
<td>74.92</td>
<td>97.33</td>
<td>77.17</td>
<td>83.79</td>
<td>7.89</td>
<td>0.0856</td>
</tr>
</tbody>
</table>
Figure 1. Plasma insulin concentrations during a 6 hour period in Holstein steers fed diets containing Soybean Meal (♦), Extruded-expelled SBMl (■), SoyPLUS® (▲), or PRO-LAK (●) (Main effect of time; P < 0.05; SEM = 1.729).
concentrations have also been reported when casein was abomassally infused in growing ruminants (Guerino et al., 1991; Davenport et al., 1995b; Ragland-Gray et al., 1997).

The results of plasma GH concentrations during the 6h sampling time periods are present in Figure 2. There was no effect treatment or time on GH concentrations (P > 0.05). Plasma growth hormone concentrations were not affected by the inclusion of different sources of RUP (P > 0.05). Results from this study agree with previous research which has shown that dietary RUP supplementation did not result in increased levels of GH (Richardel, 2004). In contrast, Davenport et al. (1990 c,d) reported increased GH concentrations with continuous infusion of arginine and ornithine into the abomasum of growing lambs and beef heifers. Davenport et al. (1995a) reported increased mean somatotropin concentrations in wether lambs fed rumen protected ornithine and arginine supplements when compared to the 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).

Source of RUP had no effect of mean plasma IGF-1 concentrations (P > 0.05). However there was an effect of time on IGF-1 concentrations, which contradicts research conducted by Gluckman et al. (1987) which showed that concentrations if IGF-1 concentrations remain stable throughout the day. Previous studies have found increased IGF-1 concentrations with varying sources and levels of RUP. Ragland-Gray et al. (1997) reported increased concentrations of IGF-1 combined with an increase in growth when casein was infused abomasally in cattle. Knaus et al. (1998) also observed that corresponding IGF-1 concentrations increased from 491 to 558 and 624 ng/mL with 2.6 and 5.2 % levels of RUP included in the diet. Davenport et al.
(1995a) also observed increases in serum IGF-1 concentrations in lambs that were fed rumen-protected AA (ornithine and arginine).
Figure 2. Plasma growth hormone concentrations during a 6 hour period in Holstein steers fed diets containing Soybean Meal (♦), Extruded-expelled SBM (■), SoyPLUS® (▲), or PRO-LAK (●) (P > 0.05; SEM = 2.879).
Figure 3. Plasma insulin-like growth factor-I concentrations during a 6 hour period in Holstein steers fed diets containing Soybean Meal (♦), Extruded-expelled SBM (■), SoyPLUS® (▲), or PRO-LAK (●) (Main effect of time; P < 0.05; SEM = 6.682).
CHAPTER 5

SUMMARY AND CONCLUSION

Summary

The objective of this study was to determine the effects of rumen undegradable protein sources on nitrogen metabolism, anabolic hormonal status, and growth Holstein steers.

Eight weaned Holstein calves approximately 6 months of age (mean BW 185.15 ± 16.16 kg) were used in a replicated 4 x 4 Latin Square designed experiment to study the effects of protein sources on performance of weaned dairy calves. Dietary treatments consisted of 16% CP diets with three sources of ruminally undegradable protein (RUP). Experimental diets were cornsilage based, with soybean meal (SBM) as the source of ruminally degradable protein (control) and 3 sources of RUP including heat treated SBM (SoyPLUS®), animal protein blend (PRO-LAK), and Extruded-Expelled SBM, all included at 45% of the dietary CP. The animals were fed their respective diets twice daily ad libitum during each 14-day adjustment period and 4-d sample collection period. Animals were housed in individual stalls (6.09 m x 4.88 m) for 14 days and in metabolism crates (152.4 cm x 76.2 cm x 121.92 cm) during the last 4 days of each experimental period. Total fecal and urine output was collected, weighed, and sub sampled for laboratory analysis of nitrogen during the 4-d collection period. On day 4 of the collection period, animals were fitted with indwelling jugular catheters. Blood samples were collected at 15-minute intervals for 6 hours for analysis of growth hormone. Also on day 4, one additional blood sample was collected, time 0, for plasma urea nitrogen (PUN) and at 30-minute intervals for analysis of IGF-I and insulin. On day 18 of each experimental period body weight, wither height, hip height, and body length were measured.
No differences (P > 0.05) were observed in DMI between treatment diets, nor did it effect any of the growth parameters measured (P > 0.05). These results indicate the different sources of RUP provided adequate amounts of metabolizable protein for growth throughout the periods. There were also no significant differences between experimental diets in the digestibility of DM, OM, NDF, ADF, or CP (P > 0.05). There were no effects (P > 0.05) of RUP protein source on nitrogen metabolism or the PUN concentrations of steers.

There were no effects (P > 0.05) of protein source on either INS, IGF-1 or GH. However, there was a time effect (P < 0.05) on concentrations of INS and IGF-1.

**Conclusion**

These data suggest that feeding diets supplemented with sources of RUP does not improve performance in 6 month old dairy calves at 16% CP. The diets consumed by the steers were formulated for 16% CP, with RUP being 45% of CP. It appeared that a masking effect was taking place in which the rumen degradable protein was mimicking the RUP source. It was concluded that feeding a TMR, using corn silage, formulated for 16% CP is more than enough protein for 6 month old dairy calves without the addition of an RUP source. More research is needed to determine if inclusion of RUP, perhaps at varying CP levels less than 16% CP may be beneficial and therefore impact growth and hormonal status.
REFERENCES


APPENDIX A

PLASMA INSULIN RADIOIMMUNOASSAY

(REF: Diagnostic Products Corporation, Coat-A-Count Insulin Kit, 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 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 18-25 hours at room temperature (15-18°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 tractor bound $\left(\frac{\text{Maximum Binding Counts}}{\text{Total}}\right) \times 100$. This should be 35-50%
4. Calculate the percentage of maximum binding $\%B/B_0 = \left(\frac{\text{Sample or Standard}}{\text{Maximum Binding}}\right) \times 100$.
5. Plot the $\%B/B_0$ 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 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 hypochlorite, phenol, at 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 hydrolyzes 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, and BLANK.
3. Add 0.010mL (10µL) of sample into corresponding vial.
4. Add 0.5mL of ENZYME RGT to all vials, mix gently, and incubate for ten minute 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 the BLANK.
7. Set wavelength at 630nm.
8. Read the STANDARD cuvettes and enter the standard value.
9. Read and record the absorbance of all the cuvettes.

Calculation

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

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. $^{125}$I-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, 12.5, 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 $^{125}$I-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 D

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 200µL of each sample INTO the glycine-HCl mixture in the appropriate tube (do NOT dribble down the side of the tube).
5. When all samples are pipette, 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 200µ (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 and 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 the 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 4 X NRS (1:105). Follow this with 200 µL of 4 X pARGG (1:45). Vortex all tubes and incubate in fridge overnight (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, 465, 775, 1550, 3100, 6200 ng.
VITA

Christopher Aaron Sissell was born in Metairie, Louisiana, to Kim and Ray Sissell. After graduating from Mandeville High School in 2000, he began his undergraduate studies in dairy science at Louisiana State University. In May, 2005, he received his Bachelor of Science degree in dairy science. After graduation he returned to Louisiana State University, in August 2005, as a graduate student in the Department of Dairy Science in the area of dairy calf nutrition. He will receive the degree of Master of Science in August of 2007. Upon completion, he plans to find a job utilizing the skills and knowledge he has learning while acquiring his master’s degree.