

EFFECT OF RACTOPAMINE ON GROWTH IN CATTLE

by

DILLON KYLE WALKER

B.S., Texas A&M University, 2002  
M.S., Tarleton State University, 2004

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Animal Sciences and Industry  
College of Agriculture

KANSAS STATE UNIVERSITY  
Manhattan, Kansas

2008

## **Abstract**

Ractopamine is a repartitioning agent that can increase muscle growth and has led to our interest in determining the mechanisms involved in enhancing muscle growth. Therefore, three studies were conducted to determine the impact of ractopamine on growth in cattle. The first experiment evaluated the impact of increasing metabolizable protein supply to finishing heifers fed ractopamine. Three different diets were fed to increase the amount of metabolizable protein reaching the small intestine, and the diets contained urea, solvent soybean meal, or expeller soybean meal as the primary supplemental protein source. From this study it was determined that increasing metabolizable protein supply above that present in typical feedlot diets containing urea and steam-flaked corn is not necessary to improve responsiveness to ractopamine. The second experiment evaluated the effect of feeding ractopamine to growing Holstein steers implanted with trenbolone acetate/estradiol. Half of the steers were implanted 28 days prior to all steers receiving ractopamine for the final 28 days; this model represents an intense implant program. The mode of action of the ractopamine and of steroidal implants was different based on their different effects on serum insulin-like growth factor (IGF)-I and longissimus expression of IGF-I mRNA. Additionally, administering a combination of the two growth promotants, based on nitrogen retention, yields a less than additive response using our model of growing Holstein steers. The third study was conducted to evaluate the differential response to ractopamine of implanted, finishing steers and heifers. Treatments were steer vs. heifer and 0 vs. 200 mg/d ractopamine fed for the final 28 days. This study attempted to address some questions generated from the previous study, which were how serum and local tissue production of IGF-I are affected by ractopamine. Ractopamine had different effects on serum IGF-I between steers and heifers and numerically increased IGF-I mRNA abundance in longissimus and biceps femoris muscles. Additionally, ractopamine impacted protein turnover differently in different muscles and changed myosin heavy chain IIA expression. The effect of ractopamine on IGF-I warrants more research. These experiments aid in our understanding of the mode of action of ractopamine in cattle.

EFFECT OF RACTOPAMINE ON GROWTH IN CATTLE

by

DILLON KYLE WALKER

B.S., Texas A&M University, 2002  
M.S., Tarleton State University, 2004

A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Animal Sciences and Industry  
College of Agriculture

KANSAS STATE UNIVERSITY  
Manhattan, Kansas

2008

Approved by:

Major Professor  
Evan Titgemeyer

## **Abstract**

Ractopamine is a repartitioning agent that can increase muscle growth and has led to our interest in determining the mechanisms involved in enhancing muscle growth. Therefore, three studies were conducted to determine the impact of ractopamine on growth in cattle. The first experiment evaluated the impact of increasing metabolizable protein supply to finishing heifers fed ractopamine. Three different diets were fed to increase the amount of metabolizable protein reaching the small intestine, and the diets contained urea, solvent soybean meal, or expeller soybean meal as the primary supplemental protein source. From this study it was determined that increasing metabolizable protein supply above that present in typical feedlot diets containing urea and steam-flaked corn is not necessary to improve responsiveness to ractopamine. The second experiment evaluated the effect of feeding ractopamine to growing Holstein steers implanted with trenbolone acetate/estradiol. Half of the steers were implanted 28 days prior to all steers receiving ractopamine for the final 28 days; this model represents an intense implant program. The mode of action of the ractopamine and of steroidal implants was different based on their different effects on serum insulin-like growth factor (IGF)-I and longissimus expression of IGF-I mRNA. Additionally, administering a combination of the two growth promotants, based on nitrogen retention, yields a less than additive response using our model of growing Holstein steers. The third study was conducted to evaluate the differential response to ractopamine of implanted, finishing steers and heifers. Treatments were steer vs. heifer and 0 vs. 200 mg/d ractopamine fed for the final 28 days. This study attempted to address some questions generated from the previous study, which were how serum and local tissue production of IGF-I are affected by ractopamine. Ractopamine had different effects on serum IGF-I between steers and heifers and numerically increased IGF-I mRNA abundance in longissimus and biceps femoris muscles. Additionally, ractopamine impacted protein turnover differently in different muscles and changed myosin heavy chain IIA expression. The effect of ractopamine on IGF-I warrants more research. These experiments aid in our understanding of the mode of action of ractopamine in cattle.

## Table of Contents

List of Figures .....	vii
List of Tables .....	viii
Acknowledgements .....	x
CHAPTER 1 - Review of Literature .....	1
Introduction.....	2
Chemical Properties of Phenethanolamines .....	2
$\beta$ -adrenergic Receptors.....	3
Receptor Desensitization.....	5
FDA Approved $\beta$ -agonists .....	6
Growth and Carcass Performance and $\beta$ -agonists .....	6
Skeletal Muscle Growth and $\beta$ -agonists.....	12
Protein Level and Ractopamine in Pigs .....	23
Steroidal Implants.....	24
Factors Affecting Response to $\beta$ -agonists.....	26
Conclusions.....	27
References.....	28
CHAPTER 2 - The Effects of Ractopamine and Protein Source on Growth Performance and Carcass Characteristic in Finishing Heifers .....	41
Abstract .....	42
Introduction.....	43
Materials and Methods .....	43
Results and Discussion.....	45
References.....	50
CHAPTER 3 - Effects of Steroidal Implantation and Ractopamine-HCl on Nitrogen Retention, Blood Metabolites, and Longissimus mRNA Expression of IGF-I in Holstein Steers .....	57
Summary .....	58
Introduction.....	59
Materials and Methods .....	59

Results .....	63
Discussion .....	64
Acknowledgements .....	67
CHAPTER 4 - Effects of Ractopamine on Growth-related Criteria in Finishing Steers and Heifers .....	78
Abstract .....	79
Introduction.....	80
Materials and Methods .....	80
Results and Discussion.....	86
References.....	94
Appendix A - .....	106
Appendix B - .....	107

## List of Figures

Figure 1.1 Phenethanolamine backbone structure .....	39
Figure 1.2 Names, structures, and classification of some phenethanolamines. ....	40
Figure 3.1 Effects of implantation and ractopamine on nitrogen retention over time in Holstein steers.....	75
Figure 3.2 Effects of implantation and ractopamine on blood metabolites over time in Holstein steers.....	76
Figure 3.3 Effects of implantation and ractopamine on <i>M. longissimus</i> mRNA expression over time in Holstein steers. ....	77
Figure 4.1 Serum IGF-I concentrations in heifers and steers fed diets with 0 or 200 mg/d ractopamine.....	105

## List of Tables

Table 2.1 Composition of the diets fed to finishing heifers .....	53
Table 2.2 Effect of ractopamine and dietary protein source on growth and carcass characteristics of finishing heifers .....	54
Table 2.3 Effect of ractopamine and dietary protein source on plasma metabolites in finishing heifers.....	55
Table 2.4 Metabolizable protein balances of heifers fed diets with different protein supplements .....	56
Table 3.1 Diet Composition .....	71
Table 3.2 Sequence of bovine specific polymerase chain reaction primers and TaqMan probes used for determination of expression of mRNA of IGF-I, and $\beta_1$ -, $\beta_2$ -, and $\beta_3$ -adrenergic receptors.....	72
Table 3.3 Effects of steroidal implantation and feeding ractopamine on nitrogen retention and diet digestibility in growing steers .....	73
Table 3.4 Effects of steroidal implantation on mRNA expression in <i>M. semimembranosus</i> and liver of ractopamine-treated steers .....	74
Table 4.1 Composition of the diet fed to finishing steers and heifers .....	98
Table 4.2 Sequence of bovine specific PCR primers and TaqMan Probes to be used for determination of expression of mRNA of IGF-I, IGFBP-3 and -5, $\beta_1$ -, $\beta_2$ -, and $\beta_3$ -adrenergic receptors, calpastatin, and myosin heavy chain IIA.....	99
Table 4.3 Effect of ractopamine on growth characteristics of finishing steers and heifers .....	100
Table 4.4 The effect of gender on blood metabolites, protein turnover, and gene expression ....	101
Table 4.5 The effect of ractopamine and gender on blood metabolites, protein turnover, and gene expression in finishing steers and heifers .....	102
Table 4.6 The effect of ractopamine on blood metabolites, protein turnover, and gene expression .....	103



Table 4.7 Effects of ractopamine and gender on RNA, DNA, and protein concentrations and  
protein:DNA, protein:RNA, and RNA:DNA ratios in the biceps femoris and LM on day 28  
.....104

## Acknowledgements

There are many who deserve thanks. I shall begin with my family. My family consists of my dad and mom, Jimmy and Janet, and my brothers, Ryon and Cody. I'm so very fortunate to have the family that I do. They have been supportive independent of my decisions during my years of uncertainty in high school and college. Without them, I could not have made it.

Evan serves as my major professor and an excellent, devoted advisor he is. Through the good and bad times, he gave it to me straight and, at the same time, was encouraging and I thank him for that. In my years at K-State, I have conducted research that is not traditionally conducted in Evan's lab, yet he was 110% willing to diverge into new avenues and I am grateful. Evan has been a great and wise mentor and friend and I hope that I can be as successful as he has been.

Brad serves on my Ph.D. committee. Research conducted by Brad is of great interest to me and, therefore, most of my research was completed in Brad's lab. I very much enjoyed working with Brad and in his lab; in addition I appreciate the support he gave me, allowing me to utilize his student workers. I thank Brad for his support during my years at K-State.

Although not directly involved with my research, Tryon Wickersham has been a positive influence in my years at K-State and in my life. Tryon has inspired me and is a great friend. With Tryon's many years of experience and success, he has obtained the greatest job an Aggie could wish for, an assistant professor position at Texas A&M University.

I also would like to acknowledge Dr. Jim Drouillard, Dr. Denis Medeiros, Dr. Barry Bradford, Cheryl Armendariz, Dr. Liaman Manedova, Dr. David Grieger, Dr. Chris Reinhardt, Dr. Larry Hollis, Dr. Ernest Minton, Dr. Jianfa Bai, Mandar Deshpande, Bill Miller, Matt Quinn, Brandon Depenbusch, Eric Loe, Jessica Starkey, Kelly Brown, Ki Yong Chung, Tim Baxa, Jaymelynn Johnson, Mandy Malone, Derek Brake, Andy Stickel, Matt Shelor, Matt May, Marisa Hands, Karl Harborth, Erin Miller, Justin Wallace, Garrett Parsons, Anna Pesta and many more for their help in allowing me to complete my Ph.D. at K-State. Additionally, I am grateful for the friends I've made outside of school in Manhattan.

# **CHAPTER 1 - Review of Literature**

## Introduction

In today's market, more pounds of lean meat are in demand from the livestock industry. Feeding livestock to produce more pounds of lean meat is the largest cost factor. For that reason, decreasing the feed required per unit of gain or product produced would improve cost effectiveness. In the last 40 years, production efficiency has been improved and accomplished by the use of growth promotants. Anabolic steroids have been used extensively. These compounds improve gain efficiency and rate of gain, thus making them cost effective. In addition, compounds called phenethanolamines or adrenergic agonists have started to be used for improving production efficiency. However, there are different classes of adrenergic agonists and within each class, each compound possesses a different potency due to the conformational structure.

## Chemical Properties of Phenethanolamines

Phenethanolamines are similar in structure to the catecholamines, epinephrine and norepinephrine (Smith, 1998). Phenethanolamines are comprised of  $\beta$ -adrenergic agonists ( $\beta$ -agonist),  $\beta$ -antagonists, and  $\alpha$ -adrenergic agonists, and some are categorized specifically within the  $\beta$ - and  $\alpha$ -adrenergic agonist subfamilies. All phenethanolamines share common features and must retain these features in order to have biological activity. Phenethanolamines must contain a substituted six-membered aromatic ring bound to a  $\beta$ -carbon in the R configuration, a positively charged nitrogen in the ethylamine side chain, and a bulky R side chain on the aliphatic nitrogen (Weiner, 1980) as shown in Fig. 1.1. However, differences in activity between phenethanolamines result from different substitutions on the aromatic ring as shown in Fig. 1.1 (A, B, and C) and substitutions of the R group adjacent to the aliphatic nitrogen. A list of different  $\beta$ -agonists is shown in Fig. 1.2. Substitution on the aromatic ring can alter activity of the compound resulting in  $\beta$ -agonists that elicit differential responses. For example, isoproterenol and dobutamine, which are categorized as catechols, have a hydroxyl group substitution at A and B in the Fig. 1.1. These substitutions lead to rapid inactivation via methylation by catecholamine O-methyl transferase (COMT; Kopin, 1985). Ractopamine and ritodrine, which are hydroxylated at the B position only, are not substrates for COMT, but are inactivated by enzymes in the intestine and liver (Smith, 1998). In addition, substituting with halogen atoms instead of hydroxyl groups, such as in clenbuterol, renders the conjugative

biotransformation enzymes ineffective while allowing these compounds to retain full binding capacity to the receptors (Morgan, 1990).

Lipophilic properties of  $\beta$ -agonists are important when looking at biological activity. Halogen substitutions on the aromatic ring such as on clenbuterol and cimaterol increase the lipophilicity of the compounds and allow them to interact with the cell membrane and, subsequently, the receptor (Witkamp, 1996). In addition, the  $\beta$ -carbon of phenethanolamines is a chiral carbon, which indicates the presences of different stereoisomers with the levorotatory stereoisomer having greater activity compared to the dextrorotatory stereoisomer (Ruffolo, 1991). Such compounds include clenbuterol, ractopamine, isoproterenol, and 6-amino [[(1-methyl-3-phenylpropyl) amino] methyl]-3-pyridine methanol dihydrochloride (L-644,969). Thus, it is apparent that all phenethanolamines are similar in structure, but small modifications, which create different structures and different  $\beta$ -agonists, can result in differences in biological activity.

### **$\beta$ -adrenergic Receptors**

There are many cell types present in a mammalian body and almost all have  $\beta$ -adrenergic receptors ( $\beta$ AR) integrated into the plasma membrane (Mersmann, 1998). The  $\beta$ AR contains approximately 400 amino acids and spans the plasma membrane 7 times. This configuration results in 3 extracellular loops and 3 intracellular loops. Ligand binding occurs in the center of the 7th transmembrane domain and interaction with the  $G_S$  protein occurs in portions of the 2, 3, and 4 intracellular loops. Three  $\beta$ AR have been identified thus far;  $\beta_1$ ,  $\beta_2$ , and  $\beta_3$ . Within species, there is approximately 40 to 50% homology between receptor subtypes (Mersmann, 1998). Across species, any given  $\beta$ AR subtype has 75% or greater homology (Pietri-Rouxel and Strosberg, 1995).

For  $\beta$ -agonists to elicit a response, they must bind to one of the  $\beta$ AR (Mersmann, 1998). Epinephrine and norepinephrine, as shown in Fig. 1.2, are known physiological  $\beta$ -agonists. Administration of synthetic  $\beta$ -agonists orally can lead to alteration in skeletal muscle and adipose tissue growth by repartitioning nutrients away from fat depots and toward muscle deposition. Beta-agonists bind to the  $\beta$ AR which results in an activated  $\beta$ AR/  $G_S$  complex. The activated  $\beta$ AR complex catalyzes the exchange of GTP for GDP on the  $\alpha$  subunit of the G protein. The  $\alpha_S$ -GTP directly activates adenylate cyclase, which catalyzes the conversion of adenosine triphosphate (ATP) to cyclic adenosine monophosphate (cAMP). Cyclic AMP binds to the

regulatory subunit of protein kinase A (PKA) in order to liberate the catalytic subunit that ultimately phosphorylates a number of intracellular proteins/enzymes (Mersmann, 1998). Phosphorylation by PKA can lead to activation of a number of proteins such as hormone sensitive lipase, which is the rate limiting step in lipolysis (Mersmann, 1998). In contrast, phosphorylation by PKA can lead to inactivation of a number of proteins such as acetyl CoA carboxylase, which is the rate limiting step in lipogenesis (Liggett and Raymond, 1993). In addition, PKA can impact gene expression by phosphorylating transcription factors such as the cAMP response element binding protein (CREB; Mersmann, 1998). This transcription factor binds to the cAMP response element in the promoter region of certain genes to activate or inactivate gene transcription. Subsequently, administration of  $\beta$ -agonists and activation of the  $\beta$ AR leads to changes in adipose tissue accretion such that lipogenesis is decreased and lipolysis is increased. In addition,  $\beta$ AR activation leads to increases in muscle growth such that protein synthesis is increased and protein degradation is decreased (Mills and Mersmann, 1995). The resulting increases in muscle growth will be discussed later in this review.

The use of  $\beta$ -agonists in the livestock industry today is to improve growth in beef cattle, lambs, swine, broilers, and turkeys; however,  $\beta$ -agonists are not equally effective in all species (Anderson et al., 1991). Overall, beef cattle and lambs show larger responses to phenethanolamines than swine, with the smallest response in chickens (Moody et al., 2000). Beef cattle and lambs show a greater response to the  $\beta_2$ -agonists, clenbuterol, cimaterol, and L-644,969, whereas swine show a lesser response (Moody et al., 2000). Additionally, differential responses between tissues within species and different muscle types are observed. Research conducted in finishing beef steers (Winterholler, 2006) and heifers (Sissom et al., 2007) demonstrated a greater abundance of mRNA for the  $\beta_2$ -AR compared to the  $\beta_1$ - and  $\beta_3$ -AR in longissimus (LM). In agreement, Silence and Matthews (1994) reported that cattle predominately have  $\beta_2$  adrenergic receptors in skeletal muscle and adipocytes. Similarly, Van Liefde et al. (1994) observed that adipose tissue in cattle contains approximately 75%  $\beta_2$ - and 25%  $\beta_1$ -adrenergic receptors. Similar results were observed in adipose tissue of lambs (Bowen et al., 1992). Ractopamine is more effective in swine than in beef cattle and is even less effective in lambs (Moody et al., 2000). In accordance, McNeel and Mersmann (1999) observed that the predominant  $\beta$ -adrenergic receptor transcript in the heart left ventricle, lungs, skeletal

muscle, and subcutaneous adipose tissue of pigs was that for the  $\beta_1$ -adrenergic receptor as measured by quantitative ribonuclease protection assays.

## **Receptor Desensitization**

As mentioned above, exposure of the  $\beta$ AR to  $\beta$ -agonist leads to increases in cAMP concentration and this response occurs within minutes of exposure (Hausdorff et al., 1990). With continued exposure to the  $\beta$ -agonist, concentrations of cAMP would be expected to increase; however, concentrations of cAMP following continual stimulation with  $\beta$ -agonists, plateau or even return to nadir concentrations (Johnson et al., 1978). A decline in cAMP concentrations in response to continuous exposure to  $\beta$ -agonist is said to occur via desensitization. Desensitization is functionally defined as an attenuation of hormonal responsiveness upon agonist stimulation (January et al., 1997).

Two separate processes can accomplish desensitization and are a result of acute or chronic exposure to a  $\beta$ -agonist. Acute  $\beta$ AR desensitization occurs with exposure in a short period of time and results in a rapid reduction in cAMP concentrations (Hausdorff et al., 1990). Removal of the desensitizing agent will attenuate desensitization within minutes. Acute  $\beta$ AR desensitization occurs via decreased agonist nonspecific responsiveness, which is a result of phosphorylation of the  $\beta$ AR by PKA or by the cAMP independent kinase,  $\beta$ -adrenergic receptor kinase ( $\beta$ ARK) or by both (Sibley et al., 1987). Phosphorylation of the  $\beta$ AR decreases the capability of the ligand-receptor complex to activate the GTP/GDP exchange on the  $\alpha$  subunit of the G protein (Sibley et al., 1987). Chronic  $\beta$ AR desensitization can occur and the exact mechanism involved is still unknown, but Hausdorff et al. (1990) suggests that chronic exposure results in loss of the receptor via down-regulation or via internalization and receptor degradation or via both. Sibley et al. (1987) reported that long-term exposure to a desensitizing agent activated  $\beta$ ARK, which initiated internalization of the  $\beta$ AR. Then phosphatases in the vesicle remove phosphates from the receptor and the receptor is not degraded. When the desensitizing agent is removed, the receptors are recycled back to the plasma membrane to maintain basal levels. In addition, Collins et al. (1989) demonstrated that long-term exposure of the  $\beta_2$ -receptor in DDT<sub>1</sub>MF-2 hamster smooth muscle cells to a desensitizing agent led to an 80% decrease in  $\beta_2$ -receptor number and a 50% decrease in mRNA abundance. Depression in mRNA abundance of the  $\beta_2$ -receptor was a result of decreased stability and not a decline in transcription rate.

The  $B_{max}$  (number of receptors) of  $\beta_2$ -receptor in the plantaris muscle was reduced by 37.7% and in the soleus muscle by 29.8% with no change in  $K_D$  in rats when fed 10 ppm cimaterol for 14 d (Kim et al., 1992). Similarly, Spurlock et al. (1994) reported  $\beta_2$ -receptor density in the middle and outer layers of subcutaneous adipose tissue was reduced by 50% in pigs weighing 78 kg fed 20 mg ractopamine/kg of diet for 24 d compared to control pigs. In the same study, ractopamine did not affect  $\beta_2$ -receptor density in skeletal muscle indicating that desensitization induced by ractopamine differs between skeletal muscle and adipose tissue. Sainz et al. (1993) demonstrated similar results in  $\beta$ -receptor density in pigs fed 20 mg ractopamine/kg of diet for 3 wk prior to harvest. In contrast, ractopamine fed for 28 d significantly increased  $\beta_2$ -receptor mRNA expression in the semimembranosus (SM) of trenbolone acetate/estradiol 17 $\beta$  (TBA/E<sub>2</sub>)-implanted crossbred yearling steers (Winterholler et al., 2007) and tended to increase  $\beta_2$ -receptor mRNA expression in the SM of implanted yearling heifers. Winterholler et al. (2006) demonstrated a tendency for  $\beta_1$ -receptor mRNA abundance to increase in LM of implanted yearling steers fed ractopamine for 28 d.

Much of the aforementioned data demonstrates that exposure of animals to  $\beta$ -agonists for an extended period of time results in a loss of responsiveness to the  $\beta$ -agonist and down-regulation of the receptor. However, this response may differ between species and between muscle and non-muscle tissues.

### **FDA Approved $\beta$ -agonists**

The addition of  $\beta$ -agonists to diets of livestock animals is increasing due to the recent FDA approval of  $\beta$ -agonists. Ractopamine-HCl, marketed as Optaflexx™, can be fed up to 400 mg per head daily to feedlot cattle for the last 28 to 42 d of the finishing period. Ractopamine-HCl, marketed as Paylean™, can be fed up to 10 mg/kg of diet per head daily to pigs for the last 20.4 to 40.8 kg of gain. Zilpaterol-HCl, marketed as Zilmax™, can be fed from 60 to 90 mg per head daily to feedlot cattle for the last 20 to 40 d of the finishing period, but with a 3 d withdrawal period prior to harvest.

### **Growth and Carcass Performance and $\beta$ -agonists**

The goal of the livestock industry is to increase meat production. This includes the use of growth promotants such as steroidal implants and  $\beta$ -agonists. As previously mentioned,  $\beta$ -



agonists are repartitioning compounds that redirect nutrient supplies away from fat deposition and toward muscle growth. Therefore, addition of  $\beta$ -agonists to the diets of meat producing animals can be beneficial.

Research on the response of pigs to ractopamine has conclusively demonstrated benefits. Armstrong et al. (2004) reported 19 and 20% increases in average daily gain (ADG) and gain efficiency (G:F) with no effect on dry matter intake (DMI) in barrows fed 10 ppm ractopamine for the final 27 d. Additionally, the authors observed a 5.2 kg gain in hot carcass weight (HCW), a 2% increase in dressing percentage (DP), a 14% increase in 10th-rib LM area, and an 8% increase in fat-free carcass lean with no change in 10th-rib fat depth and marbling score. See et al. (2004) observed 8, 8, and 16% increases in ADG, DMI, and G:F in barrows and gilts fed 20 ppm ractopamine from 71.2 to 109 kg body weight (BW; 41 d). These results were observed in pigs fed ractopamine consistently over the feeding period and in pigs stepped up on ractopamine by feeding 5 ppm for weeks 1 and 2, 10 ppm for weeks 3 and 4, and 20 ppm for weeks 5 and 6. In the same study, HCW was increased by 2.7 and 2.0 kg in the constant and step up ractopamine treatment. When HCW was used as a covariate, 10th-rib backfat depth, 10th-rib LM muscle area, and % fat-free lean were increased by 30, 12, and 6% by the constant ractopamine treatment and by 33, 15, and 8% by the ractopamine step up treatment. In contrast to the study conducted by Armstrong et al. (2004), See et al. (2004) demonstrated a 27% decrease in 10th-rib backfat depth in the constant ractopamine treatment and a 20% decrease for the step up treatment. The changes in the composition of gain with ractopamine supplementation, which were increases in muscle deposition and decreases in fat deposition, resulted in improved feed efficiency. Bark et al. (1992) demonstrated a 10 and 15% increase in ADG and G:F with no effect on DMI in genetically lean barrows fed 20 ppm ractopamine from 63 to 104 kg BW with the greatest response observed in barrows with a high lean tissue growth rate. Increases in muscle deposition in the ham, loin, shoulder, and belly in the low lean growth pigs were 12% and were 20% in the high lean growth pigs. Additionally, fat deposition in the ham and shoulder decreased by 4% in the low lean growth pigs and by 3% in high lean growth pigs. Changes in management practices, in addition to changes in genetics, have resulted in an abundance of leaner pigs when placed in the finishing period (Hollis and Curtis, 2001), and the current data suggest that genetically leaner pigs would benefit more from ractopamine feeding.

Data on the response of pigs to  $\beta_2$ -agonists has not been as conclusive. Cromwell et al. (1988) showed a 3 and 4% increase in ADG and G:F with no change in DMI in barrows and gilts fed 0.50 ppm cimaterol from 62 to 107 kg BW. Dressing percentage and HCW were increased by 1 and 2%; however, 10th-rib backfat depth was decreased by 15% and 10th-rib LM area were increased by 12%, which resulted in a 5% increase in percentage of carcass muscle. Large responses in BW and carcass gains are not observed in pigs fed cimaterol; nonetheless, changes in composition during growth occur and consequently an improvement in the lean to fat ratio is observed. Jones et al. (1985) demonstrated no change in ADG, a 7% decrease in DMI, and a 9% increase in G:F when barrows and gilts were fed 0.5 ppm cimaterol for 7 wk. Jones et al also observed a 10% increase in muscle and a 10% reduction in fat, which explains the improvements in G:F. Increases in muscle deposition were observed in the LM, biceps femoris (BF), and semitendinosus (ST), whereas the reduction in fat deposition was primarily observed at the 10th-rib. Compositional growth is altered in pigs fed cimaterol with slight improvements in BW and carcass gains, but large responses are observed in performance and carcass traits when pigs are supplemented with ractopamine.

$\beta$ -agonists are not used extensively in the poultry industry. Feeding of  $\beta$ -agonists to poultry has resulted in a range of responses. Dalrymple et al. (1984) demonstrated a 5% improvement in ADG and G:F with no effect on DMI in broiler chickens fed 1 ppm clenbuterol for 11 d. Additionally, changes in body composition were observed with a 1.5% increase in muscle deposition and a 7.5% decrease in fat deposition. Daily gains and G:F were improved by 15 and 10% when turkeys were fed 22 ppm ractopamine for 2 wk (Wellenreiter and Tonkinson, 1990). Turkeys demonstrated a larger response to the ractopamine relative to the response observed in broiler chickens administered the  $\beta_2$ -agonist, clenbuterol.

The response of cattle to ractopamine feeding is less than the response observed when they are administered a  $\beta_2$ -agonist. Schroeder et al. (2005a) demonstrated 20 and 21% increases in ADG and G:F with no effect on DMI in feedlot steers fed 20 ppm ractopamine for the final 28 to 42 d. Schroeder et al. (2005b) reported 6.4 kg more HCW and a 3% increase in LM area, but no change in 12th-rib fat thickness, kidney, pelvic, and heart fat (KPH), yield grade, and quality grade in the same study. Gruber et al. (2007) reported 15 and 17% improvements in ADG and G:F with no change in DMI in finishing steers initially implanted with 20 mg estradiol benzoate/200 mg progesterone and re-implanted 60 d later with 120 TBA/24 mg  $E_2$  when fed

200 mg ractopamine per head daily for the final 28 d. Steers demonstrated a 5.5 kg gain in HCW, a 3% improvement in LM area, a greater percentage of carcasses with yield grade 2 (45.2 vs. 30.8%), and a tendency to produce fewer carcasses with yield grade 3 (42.8 vs. 51.4%). No changes were observed in DP, KPH, adjusted fat thickness, marbling score, and quality grade. Sachtleben et al. (2006a) observed 17 and 14% improvements in ADG and G:F with no change in DMI in feedlot steers implanted with 100 mg progesterone/10 mg estradiol benzoate 64 d prior to feeding 200 mg ractopamine per head daily for the final 34 d of the finishing period. With the same steers, Sachtleben et al. (2006b) demonstrated 5.8 kg more HCW in steers fed ractopamine compared to controls and observed no effect on DP, LM area, 12th-rib fat thickness, KPH, yield grade, and quality grade. Winterholler et al. (2007) reported a 5 and 4% increase in ADG and G:F with no effect on DMI and 8 kg more HCW in yearling steers during the entire feeding period of 150, 171, or 192 d when initially implanted with 80 mg trenbolone acetate/16 mg estradiol-17 $\beta$  and re-implanted with 120 mg TBA/24 mg E<sub>2</sub> 47, 68, or 89 d prior to feeding of 200 mg ractopamine per head daily for the final 28 d. Dressing percentage, LM area, 12th-rib fat thickness, KPH, yield grade, and marbling score were not affected. However, larger responses are observed when ractopamine is fed at 300 ppm per head daily. Avendaño-Reyes et al. (2006) reported 32 and 34% increases in ADG and G:F with a 2% decrease in DMI in finishing steers initially implanted with 100 mg progesterone/10 mg estradiol benzoate 105 d prior to study and re-implanted with 200 mg TBA/28 mg estradiol benzoate 60 d prior to feeding 300 mg ractopamine per head daily for the final 33 d. Improvements in HCW and DP of 13.6 kg and 2% were observed in the same study, whereas no changes were reported in LM area and 12th-rib fat depth. Similarly, Schroeder et al. (2005a,b) observed a 26% increase in ADG and G:F, 8.3 kg more HCW, 1 and 4% increases in DP and LM area, and no change in 12th-rib fat thickness, KPH, yield grade, or quality grade in steers administered 30 ppm ractopamine. The aforementioned data in steers fed 200 mg/d ractopamine have demonstrated improvements in gains and efficiencies without affecting intake. Additionally, carcasses were heavier, which is likely due to increases in muscle deposition as reflected by increases in LM area. The 12th-rib fat depth was generally not affected, but there were changes in the yield grade distribution (Gruber et al., 2007). Increases in the dosage of ractopamine to 300 mg per steer daily resulted in a greater increase in performance compared to 200 mg/d ractopamine. Relative to 200 mg/d, 300 mg/d ractopamine tended to increase HCW, but other carcass traits did not change.

The response of finishing heifers to ractopamine has yielded results slightly different from that of steers. Schroeder et al. (2005c) observed increases in ADG and G:F of 18 and 15% when heifers were fed 20 ppm ractopamine for the final 28 to 42 d and improvements of 21 and 20% when heifers were fed 30 ppm ractopamine with no change in DMI. Schroeder et al. (2005d) reported an increase in HCW of 2.9 kg with 20 ppm ractopamine and of 5 kg with 30 ppm ractopamine fed to heifers with no changes in 12th-rib fat thickness, KPH, yield grade, or quality grade. Longissimus muscle area did not improve in heifers fed 20 ppm ractopamine, but a 4% increase in LM area was observed in heifers fed 30 ppm. Finishing heifers implanted with TBA/ E<sub>2</sub> demonstrated 11 and 14% increases in ADG and G:F with a 3% decrease in DMI when fed 200 mg ractopamine per head daily (Laudert et al., 2007). Additionally, HCW was increased by 4.6 kg and DP and LM area were increased by 1 and 2%, whereas 12th-rib fat thickness, KPH, yield grade, and marbling score were not affected by ractopamine feeding. Quinn et al. (2006) reported a 33 and 34% increase in carcass gain and carcass efficiency with no change in DMI, marbling score, yield grade, or % USDA Choice in non-implanted heifers fed 200 mg/d ractopamine for 42 d or in those fed 100 mg/d for 14 d, followed by 200 mg/d for 14 d, and 300 mg/d ractopamine for the final 14 d. These data demonstrate greater response of heifers to ractopamine when fed for the final 42 d of the finishing period rather than feeding for 28 d. Sissom et al. (2007) demonstrated a 2 and 4% improvement in ADG and G:F with no effect on DMI over the entire feeding period in finishing heifers implanted initially with 200 mg trenbolone acetate or implanted initially with 80 mg TBA/8 mg E<sub>2</sub> and re-implanted 60 d later with 200 mg TBA when fed 200 mg ractopamine per head daily for the final 28 d. Hot carcass weight was increased by 5 kg, LM area was enhanced by 3%, 12th-rib fat depth was decreased by 7%, and calculated final yield grade was decreased from 2.61 to 2.44. The decrease in 12th-rib fat depth was a response not observed in other steer and heifer studies, but it is consistent with data from pigs fed ractopamine. The heifer studies show lesser responses in growth and carcass performance than those with steers. Similar to steers, heifers fed 30 ppm ractopamine demonstrated more improvement in growth performance, HCW, and LM area than heifers fed 20 ppm ractopamine. Additionally, these data suggest that growth and carcass performance in response to ractopamine in cattle are less than those observed in pigs.

Finishing steers initially implanted with 100 mg progesterone/10 mg estradiol benzoate and re-implanted 60 d prior to the study with 200 mg TBA/28 mg estradiol benzoate

demonstrated 35 and 37% improvements in ADG and G:F with no change in DMI when fed 60 mg zilpaterol per head daily for the final 33 d (Avenidaño-Reyes et al., 2006). In the same study, steers fed zilpaterol demonstrated increases in HCW of 21.9 kg, in DP of 3%, and in LM area of 13% as well as an 18% decrease in 12th-rib fat depth. Plascencia et al. (1999) observed no change in DMI and a 37 and 28% increase in ADG and G:F in feedlot steers fed 6 ppm zilpaterol for 42 d. Hot carcass weight was increased by 13 kg, which was less than observed by Avenidaño-Reyes et al. (2006). Dressing percentage was increased by 3% with no changes in LM area, fat thickness, KPH, and marbling score. Moloney et al. (1990) reported dramatic effects of the  $\beta$ -agonist, L-644,969, on performance and carcass characteristics of finishing steers. Dry matter intake was suppressed by 10% and ADG and G:F were increased by 53 and 69% in Friesian steers fed 1.0 ppm L-644,969 for 4 wk. In addition, zilpaterol-fed steers had 23 kg more HCW, and DP and LM area were increased by 7 and 34%, whereas 10th-rib fat depth was decreased by 38.5%. Wheeler and Koochmarai (1992) demonstrated 77 and 86% increases in ADG and G:F in growing steers (initially weighing 350 kg) fed 3 ppm L-644,969 for 6 wk. Carcass performance was altered as shown by improvements in HCW by 23.3 kg and in LM area by 24% and by a 21% decrease in adjusted fat thickness. Clenbuterol administration to steers has also yielded dramatic results. Schiavetta et al. (1990) reported 35 and 25% increases in ADG and G:F in young steers (initial wt = 250 kg) fed 7 mg clenbuterol daily for 50 d. Furthermore, HCW was increased by 15 kg, LM area was increased by 28%, and yield grade was decreased from 2.6 to 1.5. In contrast, Ricks et al. (1984) demonstrated no effect of feeding 10 mg clenbuterol per head daily for 98 d to finishing Hereford steers on growth performance. Hot carcass weight was not affected by treatment, but an 11% increase in LM area and 23, 37, and 38% decreases in KPH, 12th-rib backfat thickness, and USDA yield grade were observed due to treatment with clenbuterol. The authors speculated that a high dose or a longer duration of feeding clenbuterol potentially could have limited growth responses because chronic exposure leads to receptor desensitization. Nonetheless, clenbuterol did repartition nutrients to muscle tissue as shown by an increase in LM area and decreases in KPH, 12th-rib back fat, and yield grade. Taken as a whole, these data show that feeding a  $\beta_2$ -agonist to cattle yields more dramatic improvements in growth (rate of gain, efficiency, or carcass composition) than does feeding a  $\beta_1$ -agonist.

Responses to  $\beta$ -agonists in sheep are not of the same magnitude as observed in cattle. Kim et al. (1987) reported 29 and 14% improvements in total gain and feed efficiency in wether

lambs (initial BW = 34.5 kg) fed 10 ppm cimaterol for 8 wk. Hot carcass weight in this study was increased from 24.3 to 29.5 kg when cimaterol was fed, which resulted in a 10% increase in DP. Wether lambs initially weighing 27.8 kg demonstrated 6 and 8% increases in ADG and feed efficiency when fed 10 ppm cimaterol for 7 wk (Beermann et al., 1986). Carcass traits demonstrated large changes due to cimaterol treatment as DP and LM area increased by 6 and 26% and last rib backfat thickness and KPH decreased by 48 and 35%. These data show the potent effects of cimaterol as a repartitioning agent as shown by changes in composition in wether lambs; this response was observed with minimal changes in ADG, DMI, and feed efficiency. The effect of L-<sub>644,969</sub> on performance of wether and ram lambs was less than that of cimaterol. Koohmaraie et al. (1996) reported increases in ADG and LM area of 9 and 7% and a decrease in adjusted fat thickness of 6% in 17 wk old callipyge wether and rams lambs fed 4 ppm L-<sub>644,969</sub> for 6 wk. Due to the presence of the callipyge genotype, there may have been no potential for further improvements in growth, and this might explain the lesser response to the  $\beta$ -agonist.

$\beta$ -agonists classified as  $\beta_1$  and  $\beta_2$  when fed to meat producing animals, specifically pigs, sheep, and cattle, enhance growth by repartitioning of nutrients. Repartitioning occurs when adipose tissue becomes less sensitive to nutrient supplies in circulation and muscle tissue, on the other hand, becomes more sensitive. Therefore, changes in composition of growth occur due to no change or an increase in lipolysis in adipose tissue and changes in the protein depositing machinery in muscle. Protein turnover can be affected in various ways and will be discussed later in this review. The magnitude of response to  $\beta$ -agonists depends on the  $\beta$ -agonist used and the species to which the  $\beta$ -agonist is administered.

### **Skeletal Muscle Growth and $\beta$ -agonists**

Administration of  $\beta$ -agonists to meat producing animals leads to large compositional changes in growth with lean muscle deposition increasing the most. This process occurs via repartitioning of nutrients away from fat storage and toward skeletal muscle accretion. Increases in lean muscle deposition are evident as shown by increases in individual muscle weights. Wether lambs initially weighing 17 kg demonstrated 33, 27, and 32% increases in the BF, SM, and ST when fed 10 ppm cimaterol for 7 wk (Beermann et al., 1987). Wheeler and Koohmaraie (1992) observed a 22% increase in ST weight in steers initially weighing 346 kg fed 3 ppm of

L<sub>644,969</sub> for 6 wk. Bergen et al. (1989) observed a 25% increase in ST weight of barrows initially weighing 66.4 kg fed 20 ppm ractopamine for 28 d. The BF, SM, and ST are muscles that are located in the round and have a greater distribution of fast twitch muscle fibers compared to other fiber types and, thus it appears that  $\beta$ -agonists impact them more. Some data has demonstrated effects on muscle not located in the round. Claeys et al. (1989) demonstrated a 24% increase in ST weight of lambs fed 40 ppm of clenbuterol for 28 d whereas LM weight was not affected. Similarly, Miller et al. (1988) demonstrated no change in LM weight in heifers initially weighing 393 kg at 12 months of age and fed 10 mg/d clenbuterol for 50 d. In contrast, Jones et al. (1985) demonstrated 14, 9, and 15% increases in LM area, BF weight, and ST weight when barrows and gilts were fed 0.5 ppm cimaterol for 7 wk. Bohorov et al. (1987) reported a 29% increase in LM weight of wether lambs initially weighing 29.5 kg fed 10 ppm clenbuterol for 5 wk. Bark et al. (1992) observed a 19% increase in LM weight in genetically low and high lean growth pigs fed 20 ppm ractopamine from 63 to 104 kg BW. Additionally, an increase in primal cuts has been demonstrated with  $\beta$ -agonists. Kim et al. (1987) reported 30, 24, 28, and 26% increases in the leg, loin, rack, and shoulder primal cuts in wether lambs initially weighing 34.5 kg fed 10 ppm cimaterol for 8 wk. See et al. (2004) observed an 8% increase in boneless trimmed ham in barrows and gilts fed 20 ppm ractopamine from 71.2 to 109 kg BW (41 d). Boneless trimmed shoulder, belly, loin, and tenderloin were not affected by ractopamine treatment. Although, poultry have been selected for high growth rate, responses to  $\beta$ -agonists feeding have been observed. Rehfeldt et al. (1997) reported 20, 14, 16, and 8.5% increases in the extensor hallucis longus, gastrocnemius, BF, and pectoralis profundus muscles in male and female broiler chickens fed 1 ppm clenbuterol for 3 wk. Gwartney et al. (1992) demonstrated 24.1 and 23.5 g increases in the breast and leg muscles of broiler chickens fed 1 ppm cimaterol for 14 d.

The aforementioned datum demonstrate dramatic increases in lean muscle mass and show that  $\beta$ -agonists impact muscle growth directly. Skeletal muscle contains a heterogeneous population of muscle fibers that have the ability to differentially contract and metabolize energy. The contractile speed is dependent on ATPase activity of the myosin heavy chain (MHC); thus, there are 3 muscle fiber types in bovine skeletal muscle, type I, IIA, and IIX (Chikuni et al., 2004). Energy metabolism depends on the classification of enzymes used and, therefore, muscle fibers are classified as either oxidative, glycolytic, or both oxidative and glycolytic (Pette and

Staron, 1990). Muscle fibers predominantly containing the MHC type I isoform are called slow twitch fibers due to their use during aerobic work, subsequently relying on oxidative metabolism to supply energy. When the MHC type IIA and IIX isoforms are in greater abundance, the muscle fiber is called fast twitch fiber due to its use during anaerobic work which rely on both oxidative and glycolytic metabolism to supply energy.

Demands from the livestock industry for lean red meat are increasing and because muscle fiber composition is related to animal performance in meat producing animals (Sosnicki, 1987), the use of certain stimuli are necessary to change muscle fiber composition. Administration of  $\beta$ -agonists has demonstrated changes in muscle fiber characteristics.

Rehfeldt et al. (1997) reported a 12% increase in slow twitch fiber diameter in the extensor hallucis longus, an 8% increase in diameter of fast twitch type IIA fibers in the extensor hallucis longus muscle, and a 9% increase in diameter of fast twitch type IIA fibers in the gastrocnemius muscle in broiler chickens fed clenbuterol. Response to clenbuterol resulted in changes of the percentage of fast twitch type IIA fibers from 41.5 to 36% and of fast twitch type IIB fibers from 38 to 44% in the extensor hallucis longus muscle. Conversely, Aalhus et al. (1992) reported no change in percentage and diameter of red (type I slow twitch) muscle fibers in ST and psoas major of pigs (64 kg) fed 20 ppm ractopamine until slaughter BW of 100 kg was reached. Fiber diameter of intermediate fibers (type IIA and IIX fast twitch) in ST and psoas major increased by 24% due to ractopamine; however, percentage of intermediate fibers decreased from 12.9 to 10.4%. Fiber diameter of white fibers (type IIB fast twitch) in ST and psoas major increased by 17% and frequency increased from 72.8 to 77.3% due to ractopamine feeding. The diameter of both intermediate and white muscle fibers increased; nevertheless, the distribution shifted to more white muscle fibers. Oksbjerg et al. (1994) demonstrated no change in cross sectional area of slow twitch, fast twitch IIA, and fast twitch IIB in LM and BF of pigs initially weighing 25 kg and fed 3 ppm salbutamol until slaughter at 90 kg (avg. 92 d). However, frequency of slow twitch fibers decreased from 12.3 to 10.7%, fast twitch IIA fibers decreased from 9.5 to 5.5%, and fast twitch IIB fibers increased from 78.2 to 83.8% in LM, whereas in the BF frequency of slow twitch fibers decreased from 23.6 to 19.5%, fast twitch IIA fibers decreased from 13.3 to 7.8%, and fast twitch IIB fibers increased from 63.2 to 72.7%. These data support Aalhus et al. (1992) demonstrating the conversion of fast twitch IIA fibers to fast



twitch IIB fibers. Additionally, the latter study demonstrates the ability of salbutamol to convert slow twitch fibers to fast twitch fibers.

The data presented shows that administration of  $\beta$ -agonists either increases the size of each fiber type or converts slow fibers to fast glycolytic fibers. Changes in the size of the fiber can be attributed to alterations in protein turnover that will be discussed later in this review. A muscle is classified based on the fiber type distribution. The fiber type profile in each muscle is determined by the neural input. If the neural input is changed such as using cross-reinnervation, then the fiber type profile will change (Pette and Staron, 1997). For instance, cross-reinnervation of a fast twitch muscle with a slow nerve converts the fast twitch muscle to a slow twitch muscle. Conversely, cross-reinnervation of a slow twitch muscle with a fast nerve converts the slow twitch muscle to a fast twitch muscle (Pette and Staron, 1997). Additionally, changes in expression of MHC isoform can change the profile of a fiber and could provide an *in vivo* measure of changes in protein turnover. Depreux et al. (2002) demonstrated 20, 33, and 32% decreases in MHC IIA mRNA expression on d 14, 28, and 42, and 42 and 48% decreases in MHC IIX mRNA expression on d 28 and 42, whereas MHC IIB mRNA expression increased by 40 and 29% on d 28 and 42 with no change in MHC IA mRNA expression on d 14, 28, and 42 in ST and LM of pigs initially weighing 85 kg when 20 ppm ractopamine was fed. Additionally, pigs fed 60 ppm ractopamine demonstrated 36 and 49% decreases in MHC IIA and 34 and 59% decreases in MHC IIX mRNA expression on d 28 and 42, whereas MHC IIB mRNA expression increased by 35 and 60% on d 28 and 42. Gunawan et al. (2007) reported no change in type I MHC gene expression, a gradual decrease of type IIA MHC gene expression to wk 1 with an increase to control levels by 4 wk, a decrease in type IIX MHC gene expression throughout the feeding period, and an increase in type IIB MHC gene expression in pigs (initially weighing 90 kg) fed 20 ppm ractopamine for 4 wk. In steers, Smith et al. (1989) demonstrated an increase in fast twitch myosin light chain (MLC) 1/3 mRNA expression in response to 0.72 mg/kg BW ractopamine for 56 d. These data indicate that changes in gene expression of MHC and MLC are induced by  $\beta$ -agonist feeding and could indicate either changes in fiber type profile or changes in protein accretion.

Data reported on myofibril morphology in ruminants have demonstrated results similar to that in pigs. Beermann et al. (1987) observed increases in type I fiber area in superficial ST, deep medial ST, and LM of 32, 29, and 13% and in type II fiber area in superficial ST, deep

medial ST, LM, SM of 29, 29, 15, and 55% in wether lambs fed 10 ppm cimaterol for 12 wk. The frequency of type I fibers decreased from 10.7 to 3.7% in medial ST, 3.6 to 0.4% in superficial ST, 3.5 to 0% in SM, and 0.7 to 0.2% in LM due to cimaterol feeding when compared to control lambs. A decrease in the percentage of type I fibers resulted in an increase in the frequency of type II fibers in all muscles. In contrast, Kim et al. (1987) observed no change in type I fiber area in LM and ST of wether lambs fed 10 ppm cimaterol for 8 wk. Nonetheless, type II fiber area was increased by 50 and 51% in LM and ST of lambs fed cimaterol compared to controls. Vestergaard et al. (1994) reported an increase in fiber area in type I fibers of 31% and in type IIB fibers of 69% in LM of Friesian bulls (initially weighing 162 to 407 kg) when fed 0.06 ppm cimaterol for 90 d. Frequency of type I fibers in LM decreased from 24.0 to 20.4% and type IIA fibers decreased from 24.2 to 8.6%, whereas frequency of type IIB increased from 51.8 to 71.1%. Additionally, in ST, type I and IIA fiber areas were not affected by cimaterol, but type IIB fiber area increased by 22%. The percentage of IIA decreased from 24.3 to 6.7% and of IIB increased from 61.8 to 81.6% in the ST. Wheeler and Koohmaraie (1992) demonstrated no change in fiber distribution of  $\beta$ R ( $\beta$ -red, slow twitch),  $\alpha$ R ( $\alpha$ -red, fast twitch IIA), and  $\alpha$ W ( $\alpha$ -white, fast twitch IIB) in the LM when steers (initially weighing 350 kg) were fed 3 ppm L<sub>644,969</sub> for 6 wk. However,  $\beta$ R fiber area decreased by 41% and  $\alpha$ R and  $\alpha$ W fiber area increased by 24.5 and 7.6%. Miller et al. (1988) demonstrate a 19% increase in type II fiber diameter with no change in type I diameter and frequency of type I and II in the LM of heifers fed 10 mg/d clenbuterol for 50 d. Gonzales et al. (2007) observed no change in the percentage of type I and type II fibers in the LM of cull cows, initially weighing 370 to 418 kg, when fed 15 ppm ractopamine for 35 d. In contrast, type I fiber area and diameter were increased by 31 and 15%, whereas type II fiber area and diameter were not affected by ractopamine. In the latter study, MHC isoforms were detected by immunocytochemistry, but type IIX and IIB were not detected. Chikuni et al. (2004) did not detect type IIB mRNA from bovine muscle using real-time PCR, but did detect type IIX. Pig skeletal muscle contains 4 types, I, IIA, IIX, and IIB.

With the data at hand,  $\beta$ -agonists can alter muscle composition, alter expression profile of MHC, and increase of the diameter of each muscle fiber in pigs and cattle with little impact on poultry. Myosin heavy chain expression changes such that type I, IIA, and IIX are down-regulated and type IIB is up-regulated. The distribution of muscle fibers is changed such that the fast, glycolytic and/or oxidative fibers increase, whereas slow, oxidative fibers decrease in

percentage. In general, all muscle fiber types increase in size, but predominately the fast, type II fibers are impacted such that area and frequency increase.

Improvements in muscle growth are observed when meat producing animals are fed  $\beta$ -agonists. As the previous section discussed, muscle composition is changed to favor the increase in lean muscle mass. The increase in size or hypertrophy of the muscle fiber (cell) is due to changes in protein turnover: increases in protein synthesis, decreases in protein degradation, or both. In addition, blood urea-N will decrease in animals fed  $\beta$ -agonists indicating improved utilization or decreased catabolism of amino acids. Dunshea and King (1994) reported a 5% decrease in plasma urea-N in gilts fed 20 ppm ractopamine for 22 d. Additionally, Eisemann and Bristol (1998) demonstrated a 19% decrease in plasma urea in 487 kg steers fed 80 ppm ractopamine for 15 d. See et al. (2004) reported decreases in plasma urea-N of 27, 22, and 14% on d 7, 21, and 35 in pigs fed 11.7 ppm ractopamine. These data demonstrate decreases in plasma urea-N with  $\beta$ -agonist feeding which results from increases in retained nitrogen in skeletal muscle. Improvements in nitrogen retention are associated with increased uptake of amino acids into the muscle. Data reported by Byrem et al. (1998) demonstrated 37, 66, 61, 52, 47, 59, 55, 45, 45, and 46% increases in net flux of serine, tyrosine, arginine, histidine, lysine, phenylalanine, threonine, isoleucine, leucine, and valine into the hind limb of steers (initially weighing 250 kg) when arterially infused with 0.5  $\mu$ g cimaterol/ min for 14 d. Enhanced uptake of amino acids resulted from the direct action of cimaterol on muscle accretion.

The use of isotopes can also give estimates of fractional protein turnover rates when animals are administered  $\beta$ -agonists and can further elucidate the direct impact  $\beta$ -agonists have on muscle growth. Helderich et al. (1990) reported a 55% increase in fractional synthesis rate of  $\alpha$ -actin, which constitutes 23% of myofibrillar protein, in the LM of pigs when administered 20 ppm of ractopamine for 21 d. Additionally,  $\alpha$ -actin mRNA expression and translational activity was enhanced 2-fold in pigs fed ractopamine. Translational activity of other proteins was enhanced, and it can be concluded that ractopamine not only impacts gene expression, but also improves mRNA stability and production of the final protein product. Similarly, Grant et al. (1993) reported 41 and 62% increases in  $\alpha$ -actin mRNA expression in the LM muscle of pigs fed 20 ppm ractopamine for 2 and 4 wk. Using ELC<sub>5</sub> (subclone of L6 myoblast) cell line, Anderson et al. (1990) demonstrated 12, 27, 18, 34, and 24% increases in [<sup>35</sup>S]-methionine incorporation into total protein of myotubes after 4, 24, 48, 72, and 96 h of incubation with 1  $\mu$ M ractopamine.

Similarly, increases in [<sup>35</sup>S]-methionine incorporation were observed in 43-kDa proteins that represent either  $\alpha$ -actin, creatine kinase, other sarcoplasmic proteins, or MHC proteins. Bergen et al. (1989) reported that fractional protein synthesis and degradation rates were 4.4%/d and 3.4%/d for control and 6.1%/d and 4.9%/d in the ST of pigs fed 20 ppm ractopamine for 21 to 35 d. Subsequently, fractional accretion rates were improved by 20% with ractopamine feeding and can be attributed to increases in both synthesis and degradation. These data demonstrate that ractopamine-enhanced improvements in muscle growth are mediated, in part, by increases in protein synthesis.

Claeys et al. (1989) demonstrated a 9.4%/d fractional synthesis rate in the brachialis muscle of lambs fed 40 ppm of clenbuterol for 28 d compared to only 6.1%/d for control lambs. Fractional degradation rates were not affected by clenbuterol treatment in that study. Young et al. (1990) demonstrated a 10 to 12% increase in [<sup>3</sup>H]-leucine incorporation into myosin heavy chain and a 10 to 15% decrease in protein degradation rates in primary muscle cells prepared from broiler chicken leg incubated with 1  $\mu$ M cimaterol for 7 d. These data demonstrate the combined result of increases in protein synthesis and of decreases in protein degradation when primary muscle cells isolated from chickens are incubated with clenbuterol. Wheeler and Koohmaraie (1992) demonstrated a 27.1% decrease in fractional protein degradation rates and a numerical increase in fractional protein synthesis rates after 3 wk in steers fed 3 ppm of L<sub>644,969</sub>. In contrast, Benson et al. (1991) demonstrated no change during 2 h incubations in [<sup>14</sup>C]-phenylalanine incorporation into soleus muscle removed from rats fasted and treated with 2 ppm clenbuterol for 2 d. No change in concentration of tyrosine in the media after 2 h incubation was observed. However, concentration of 3-methylhistidine was significantly lower in the media at 0 and 2 h of incubation of the treated muscle compared to control, indicating a reduction in protein degradation. Release of tyrosine from the muscle reflects breakdown of total protein, whereas release of 3-methylhistidine reflects breakdown of myofibrillar proteins such as actin and myosin. Therefore, clenbuterol treatment to fasted rats had no effect on protein synthesis, but decreased myofibrillar protein degradation. Reeds et al. (1986) reported no change in muscle protein synthesis, but demonstrated decreases in protein degradation of 55, 39, 19, and 32% in the gastrocnemius and of 55, 25, 30, and 26% in the soleus muscle of rats on d 4, 11, 21, and 25 when administered 0.2 ppm clenbuterol.

$\beta$ -agonists greatly influence protein metabolism via suppression of protein degradation. Three major protein catabolic pathways are found in muscle: a lysosomal pathway (cathepsin B, D, and L, and their inhibitor, cystatin), a calcium-dependent pathway ( $\mu$ -calpain, m-calpain, and their inhibitor, calpastatin), and an ATP-dependent ubiquitin-proteasome pathway. The calcium-dependent pathway or calpain system is key to skeletal muscle turnover. The calpains are intracellular proteins found in the muscle fiber and are localized around the Z disk (Goll et al., 2002). The calpains initiate protein degradation in the muscle via cleavage of desmin, vinculin, talin, dystrophin, spectrin, titin, nebulin, troponin T and I, tropomyosin, and actinin which are proteins associated with the Z disk (Goll et al., 1998). Actin and myosin constitute a majority of myofibrillar proteins and are not degraded by the calpain system. Due to disassociation of the Z disk and release of other proteins, actin and myosin disassociate and are degraded by the lysosomal and/or proteasome system (Goll et al., 1998). Calpastatin controls this process via down-regulation of  $\mu$ -calpain and m-calpain which lead to decreases in protein degradation. Thus, administration of  $\beta$ -agonists could inhibit these proteolytic systems resulting in less protein being degraded. Koohmaraie et al. (1991) demonstrated a 63% increase in calpastatin activity in the BF of lambs fed 4 ppm L<sub>644,969</sub> for 6 weeks. Activity of  $\mu$ -calpain, m-calpain, cathepsin B or L, and cystatin were not affected by  $\beta$ -agonist treatment. In addition, Parr et al. (1992) observed increases in activity of m-calpain and calpastatin by 27 and 76% in LM of steers fed 1.5 ppm cimaterol for 16 weeks. In addition, mRNA levels of m-calpain and calpastatin were increased by 30 and 96% due to cimaterol treatment. The authors were the first to report simultaneous up-regulation of m-calpain and calpastatin due to  $\beta$ -agonist feeding and suggested that this may be the physiological response observed when  $\beta$ -agonist are used. Forsberg et al. (1989) reported 58, 57, and 52% decreases in activity of m-calpain,  $\mu$ -calpain, and calpastatin in skeletal muscle (BF, gastrochemius, and SM) of rabbits fed 10 ppm cimaterol for 35 d. Activity of cathepsin B and D were not affected by cimaterol treatment. Reduced activity of calpastatin was not expected due to down-regulation of both calpains. However, the authors mention that calpastatin activity that was present was enough to depress calpain activity.

The ubiquitin-proteasome system is responsible for myofibrillar proteolysis after myofibrillar proteins have been cleaved from the sarcomere by the calpain system (Attaix et al., 1998). When proteins are degraded via the ubiquitin-proteasome system, they are first tagged by the 14-kDa E2 and E3- $\alpha$  protein, and the tagged proteins are then degraded by the 20S

proteasome (Yimlamai et al., 2005). In a study conducted by Yimlamai et al. (2005), a hindlimb unweighting model was used to simulate muscle atrophy. The authors demonstrated in mice subjected to hindlimb unweighting plus administration of 0.9 mg/d clenbuterol via drinking water for 14 d an attenuation of hindlimb unweighting induced atrophy and decreased ubiquitin conjugates only in the fast plantaris and tibialis anterior, but not in the soleus muscle compared to rats subjected to hindlimb unweighting. These findings support the notion of  $\beta$ -agonist acting predominantly on protein catabolism and, in addition, these data show that clenbuterol only affected the proteolytic system in fast twitch muscles and not in slow twitch muscle.

Based on the evidence above,  $\beta$ -agonists alter protein metabolism such that protein synthesis is increased, protein degradation is decreased, or both. Ractopamine increases protein synthesis as shown by increased myofibrillar protein mRNA and protein production, whereas cimaterol and clenbuterol,  $\beta_2$ -agonists, mostly decrease protein degradation via down-regulation of two proteolytic systems; however, some data on administration of  $\beta_2$ -agonists have demonstrated increases in protein synthesis in addition to decreases in degradation.

The aforementioned data demonstrate that  $\beta$ -agonist administration results in conformational changes in the muscle by changing muscle fiber type distribution. Additionally, treatment with  $\beta$ -agonists leads to increases in protein accretion via inhibition of protein degradation, although some research demonstrated an additional increase in protein synthesis. Increases in protein accretion in response to  $\beta$ -agonists can be limited if the amount of DNA in each muscle fiber is not increased. Muscle hypertrophy increases the area of cytosol associated with each nuclei (increasing the protein:DNA ratio), placing a greater demand on nuclei to initiate gene expression of myofibrillar and sarcoplasmic proteins. Therefore, increasing DNA content in the muscle fiber could lessen the demand on the nuclei and would not limit the growth of the muscle fiber. However, myofibril nuclei are mitotically inactive and therefore, muscle fibers require another source of DNA during postnatal muscle growth. Satellite cells serve as the main source of DNA for muscle fibers during postnatal muscle growth (Moss and LeBlond, 1971). Satellite cells are mono-nucleated cells that lie in close proximity to a muscle fiber and are located between the basal lamina and sarcolemma (Mauro, 1961). Satellite cells found in skeletal muscle were first discovered 45 years ago (Mauro, 1961). In an unperturbed state, satellite cells are quiescent. When activated by injury, stretch, exercise, or some signal, satellite cells undergo asymmetric division resulting in potentially half of the new daughter cells

undergoing differentiation and subsequently fusing with existing muscle fibers to aid in muscle regeneration or to sustain muscle hypertrophy (Hawke and Garry, 2001; Zammit and Beauchamp, 2001). Therefore, these satellite cells serve as a reserve source of DNA that is needed for muscle repair or muscle growth.

Grant et al. (1990) demonstrated a 200% increase in the number of nuclei in satellite cell culture (isolated from embryonic chick breast muscle) incubated with 1  $\mu$ M ractopamine for 48 h. Ractopamine increased satellite cell activity, thus changing the DNA content in the cell. Grant et al. (1993) observed an 18% increase in DNA content with no change in DNA concentration in the LM of pigs fed 20 ppm ractopamine for 4 wk. The increase in DNA content was enough to maintain the protein:DNA ratio. Forsberg et al. (1989) reported 77, 55, and 54% increases in DNA content in BF, extensor digitorum longus, and gastrocnemius muscle of rabbits fed 10 ppm cimaterol for 35 d. In contrast, Gonzales et al. (2007) demonstrated no change in fiber-associated nuclei and satellite cell number in satellite cells isolated from cull cows fed between 15 and 16.5 ppm ractopamine for 35 d prior to harvest. Similarly, Beermann et al. (1987) showed no change in total DNA content with a subsequent decrease in DNA concentration in ST in lambs fed 10 ppm of cimaterol for 7 wk. O'Connor et al. (1991) demonstrated 42 and 25% reductions in DNA concentrations in the ST of ram lambs fed 10 ppm cimaterol for 3 and 6 wk. With 34 and 19% increases in ST weight at 3 and 6 wk of cimaterol feeding, protein:DNA ratio was increased by 76 and 51%. Additionally, Rehfeldt et al. (1997) demonstrated no change in DNA content in the extensor hallucis longus muscle of male and female broiler chickens fed 1 ppm clenbuterol for 3 wk. Gwartney et al. (1992) demonstrated 14 and 18% decreases in DNA concentration of the breast and leg muscles of broiler chickens fed 1 ppm cimaterol for 14 d.

The current dogma on the response of skeletal muscle satellite cell proliferation to  $\beta$ -agonist treatment is no change in activity or DNA content of the muscle fiber. Any stimulus ( $\beta$ -agonist) that increases cAMP concentrations in the cell is generally considered to inhibit cell proliferation (Dumont et al., 1989). However, some of the data presented above demonstrated DNA accretion in skeletal muscle cells exposed to  $\beta$ -agonist treatment. Dumont et al. (1989) reviewed the impact cAMP has on cell proliferation and concluded that other cell types can stimulate cell proliferation via cAMP. It is possible that non-myogenic cell types found within

muscle (e.g., fibroblasts) respond to  $\beta$ -agonist administration by accreting DNA and make it appear that cell proliferation is enhanced in satellite cells.

The previous data demonstrate clearly that  $\beta$ -agonists increase muscle growth. Whether the actions of  $\beta$ -agonists are direct or indirect has not been extensively studied. However, data reported by McElligott and Chaung (1988) and Young et al. (2002) demonstrate that the addition of serum from rats and chickens treated with clenbuterol to primary cell cultures resulted in no change in [<sup>3</sup>H]-thymidine incorporation, protein turnover rates, and cAMP production when compared to control cultures. These data suggest that responses to  $\beta$ -agonists could be direct. Steroidal implants are utilized in the feedlot industry to improve muscle growth similar to  $\beta$ -agonists. Johnson et al. (1996a,b; 1998a,b) demonstrated that administering implants to feedlot cattle improves muscle growth that is mediated through increases in liver secretion and skeletal muscle production of insulin-like growth factor (IGF)-I. The role of IGF-I in the response to steroidal implants will be discussed later in this review. Insulin-like growth factor-I plays a pivotal role in postnatal muscle growth and myogenesis.

Beermann et al. (1987) reported 46.5 and 21.5% decreases in serum IGF-I concentrations in lambs fed 10 ppm cimaterol for 6 and 12 wk compared to lambs not fed cimaterol. Additionally, Sainz et al. (1990) reported that soleus and extensor digitorum longus muscle samples collected from rats treated with 0.9 mg/d cimaterol for 11 d had less response to IGF-I in culture compared to control. These data suggest that IGF-I may not play a role in  $\beta$ -agonist induced muscle growth. In contrast, O'Connor observed no change in serum IGF-I concentrations in ram lambs initially weighing 34.5 kg and fed 10 ppm cimaterol for 3 or 6 wk. Dunshea and King (1994) noted a similar lack of change in IGF-I in pigs fed 20 ppm ractopamine for 22 d. Plasma IGF-I concentrations were not affected by feeding of 1.5 ppm cimaterol to growing Friesian steers (12 wk old weighing 92 kg) for 16 wk (Dawson et al., 1993) or by feeding 0.4 ppm clenbuterol to ewe lambs initially weighing 20.4 kg for 80 d (Young et al., 1995). These data show differential circulating IGF-I responses to  $\beta$ -agonist treatment. Grant et al. (1993) demonstrated no change in IGF-I mRNA expression in the liver and LM of pigs fed 20 ppm ractopamine for 4 wk. Yimlamai et al. (2005) using a hindlimb unweighting model to simulate muscle atrophy, demonstrated in mice subjected to hindlimb unweighting plus administration of clenbuterol for 14 d a decrease in IGF-I content in the fast plantaris but not in the tibialis anterior muscle compared to rats subjected to only hindlimb unweighting. In contrast,



Awede et al. (2002) demonstrated a 5-fold increase in IGF-I mRNA expression, a 8-fold increase in IGF binding protein (IGFBP)-4 mRNA expression, and a 5-fold increase in IGFBP-5 mRNA expression in the soleus muscle of 3 month old rats administered 10 ppm clenbuterol in the drinking water for 3 d. Additionally, muscle IGF-I content transiently increased by d 1 to d 5, whereas serum concentrations of IGF-I decreased due to clenbuterol. The early induction of muscle IGF-I content relative to increases in mRNA expression of IGF-I indicate clenbuterol initially impacts mRNA translational rate and/or posttranslational modifications in skeletal muscle. The reason for decreases in circulating IGF-I is not known, but the action of clenbuterol seems to preferentially to be on skeletal muscle. Additionally, the role of increased IGFBP-4 when clenbuterol is administered is unclear, but increases in IGFBP-5 have been implicated in inhibition of cell proliferation via IGF-I independent action. The IGFBP-5 is produced and secreted by porcine embryonic myogenic cells, translocated back into the cell and into the nucleus, and inhibits cell proliferation (Kamango-Sollo et al., 2005). Potentially, clenbuterol could elevate local production of IGF-I resulting in muscle hypertrophy; however the elevation of IGFBP-5 mRNA could suppress satellite cell activation and proliferation limiting the action of IGF-I to changes in protein turnover.

Administration of  $\beta$ -agonists has exhibited pronounced effects on muscle deposition. Slow to fast twitch fiber conversion or increases in slow and fast twitch fiber diameter is observed with  $\beta$ -agonists; however, in general, the fast glycolytic fibers are impacted the most when  $\beta$ -agonists are fed. Changes in protein turnover are observed and, although some research demonstrated increases in protein synthesis, protein degradation is primarily affected by  $\beta$ -agonist treatment. The impact of  $\beta$ -agonists on skeletal muscle satellite cell proliferation and subsequently DNA accretion is still unclear, but, in part,  $\beta$ -agonists seem not to stimulate cell proliferation. Additionally, it is unclear to what extent, if any, IGF-I mediates the effects of  $\beta$ -agonists on muscle growth.

### **Protein Level and Ractopamine in Pigs**

Lysine is typically the first limiting amino acid in swine diets, and supplementation of crystalline lysine has become a popular practice. Baker et al. (1975) and Easter and Baker (1980) demonstrated that decreasing crude protein in the diet by 2%, which resulted in decreased pig performance, can be attenuated by the addition of 0.15% L-lysine-HCl. Subsequently, more

crystalline amino acids are being added to replace a portion of soybean meal in the diet to insure adequate amino acids are available, to decrease cost, and to decrease fecal N excretion (Carter et al., 1996).

With the increased demand for lean meat production, swine producers have begun to incorporate the  $\beta$ -agonist, ractopamine, into their diets. Improving muscle growth with the addition of ractopamine could change the lysine requirement. Apple et al. (2004) demonstrated linear increases in ADG, G:F, LM depth and area, fat free lean yield, and ham yield as lysine:metabolizable energy (lysine:ME) ratio increased from 1.7 to 3.1 g/Mcal and in pigs fed 10 ppm ractopamine for 28 d. Additionally, 10th-rib fat depth linearly decreased as lysine:ME increased. Similarly, Webster et al. (2002) demonstrated linear increases in ADG, G:F, BW, HCW, lean percentage, and loin eye area as dietary lysine increased from 0.8 to 1.4% and as ractopamine increased in the diet from 0 to 10 ppm for 28 d. Lysine requirements are increased to 1.4% when ractopamine is included in the diet and is greater than the lysine requirement of 1.1 to 1.2% in pigs not fed ractopamine.

### **Steroidal Implants**

The use of growth promoting compounds in meat producing animals has become an important strategy in the feedlot industry. Anabolic steroids are growth promotants and have been utilized for nearly 45 yr (Johnson et al., 1996a). Trenbolone is a synthetic androgen that is similar in structure to testosterone (Anderson, 1991). Trenbolone acetate is 10 to 50 times more potent in eliciting responses than testosterone and, therefore, is used instead of testosterone (Bouffault and Willemart, 1983). Hayden et al. (1992) demonstrated 3% increases in BW and 7% increases in ADG in crossbred steers initially weighing 302 to 312 kg and implanted with 300 mg TBA for 80 d. In the same study, steers implanted with 24 mg E<sub>2</sub> demonstrated 7 and 20% increases in BW and ADG. Additionally, implanting with 300 mg TBA plus 24 mg E<sub>2</sub> resulted in 11 and 37% improvements in BW and ADG. Implantation with TBA plus E<sub>2</sub> resulted in a synergistic response compared to the response in E<sub>2</sub> implanted steers which was greater than steers implanted with TBA. Bartle et al. (1992) reported 18 and 10% increases in ADG and G:F in finishing steers implanted with 140 mg TBA/28 mg E<sub>2</sub> for 140 to 168 d compared to control steers and 10 and 7% increases in ADG and G:F when compared to steers implanted with 30 mg E<sub>2</sub>. Daily gains and G:F in steers implanted with 140 mg TBA alone were not different from

control steers. In the same study, steers implanted with 140 mg TBA/28 mg E<sub>2</sub> demonstrated a 5% increase in LM area and 7 and 5% decreases in KPH and marbling score compared to control and to TBA implanted steers. Finishing steers implanted with 120 mg TBA/24 mg E<sub>2</sub> demonstrated 82, 34, and 25% increases in carcass protein gain over control steers at d 40, 115, and 143 of implantation (Johnson et al., 1996a). These data suggest that the additional protein accretion occurs primarily in the first 40 d after implantation. Taken together, the combined TBA/E<sub>2</sub> implant yields greater responses in feedlot steers compared to TBA or E<sub>2</sub> alone.

Due to the anabolic effects of the combined use of TBA and E<sub>2</sub> on protein accretion, the exact mechanism by which these effects are mediated had been studied and IGF-I has been observed to be involved (Hayden et al., 1992). Johnson et al. (1996b) demonstrated 9, 13, and 19% increases in circulating IGF-I concentrations in steers implanted with 120 TBA/24 mg E<sub>2</sub> for 21, 40, and 115 d. The activity and half-life of IGF-I is under control of IGFBP and, therefore, was measured in serum samples. Implanted steers demonstrated greater IGFBP-3 concentrations on d 21 and 40 compared to non-implanted steers. The increases in serum IGF-I and IGFBP-3 occurred at the same time when protein accretion was the greatest in implanted steers. Additionally, serum was collected from implanted and non-implanted steers throughout the experiment and was used to determine its mitogenic activity. Satellite cell proliferation was increased by the addition of serum from implanted steers when compared to the proliferation rates of cells cultured with serum from non-implanted steers. Serum concentrations of IGF-I and to a greater extent IGFBP-3 increased due to implantation resulting in greater mitogenic activity. Johnson et al. (1998a) observed a 150% increase in hepatic IGF-I mRNA abundance in lambs implanted with 40 mg TBA/8 mg E<sub>2</sub> for 40 d. In the same study, finishing steers implanted with 120 mg TBA/ 24 mg E<sub>2</sub> for 40 d demonstrated a 68% increase in IGF-I mRNA expression in the LM. Similarly, increases in IGF-I in the liver and SM (White et al., 2003) and in the LM (Pampusch et al., 2003; Dunn et al., 2003) have been demonstrated in steers implanted with the combination of TBA/E<sub>2</sub>. Increases in hepatic IGF-I mRNA abundance coincide with elevated circulating IGF-I concentrations after implantation. Localized production of IGF-I in muscle can initiate a response through an autocrine or paracrine action resulting in improved muscle accretion. Additionally, IGF-I, being a potent mitogen, can stimulate proliferation and fusion of satellite cells into existing muscle fibers, increasing the DNA content of the cell. Skeletal muscle satellite cells isolated from yearling steers implanted with TBA/E<sub>2</sub> for 31 d had greater

[<sup>3</sup>H]-thymidine incorporation rates 24 and 34 h post plating, 43% more cells at 72 h post plating, and greater number of myotube nuclei than satellite cells isolated from steers not implanted (Johnson et al., 1998b).

Collectively, these data show that implantation with both TBA and E<sub>2</sub> yield synergistic responses compared to implantation with TBA or E<sub>2</sub>. The combined use of TBA/ E<sub>2</sub> yields improvements in protein accretion while simultaneously increasing serum IGF-I and IGFBP-3 concentrations, IGF-I mRNA expression in the liver, SM, and LM, and stimulating skeletal muscle satellite cell proliferation in vitro.

### **Factors Affecting Response to $\beta$ -agonists**

Differences between genders in growth performance are apparent and, therefore, differences between gender in response to  $\beta$ -agonists may be expected. Rehfeldt et al. (1997) demonstrated improvements in ADG and feed efficiency of 8 and 7% in male broiler chickens and of 5 and 4% in female broiler chickens fed 1 ppm clenbuterol for 3 wk. Male chickens demonstrated 11 and 4% increases in protein and fat gain, whereas female chickens showed a 16% increase in protein gain and a 3% decrease in fat gain. It is apparent that differences in response to ractopamine exist between genders. Males demonstrated larger improvements in ADG, G:F, and carcass muscle gain, whereas females showed small ADG and G:F responses, but a larger change in carcass composition. In swine, barrows grow faster than gilts; however, gilts are more efficient in lean tissue deposition than barrows (Friend and MacIntyre, 1970). Therefore, the addition of  $\beta$ -agonists to the diet might benefit barrows to a greater extent than gilts. See et al. (2004) observed a 10% increase in ADG, a 2% increase in DMI, and a 10% increase G:F in barrows fed ractopamine, whereas gilts demonstrated a 2% increase in ADG, a 13% decrease in DMI, and a 19% increase in G:F in gilts fed ractopamine compared to control. Additionally, gilts demonstrated 3 and 6% improvements in fat-free lean and boneless trimmed loin compared to barrows. The data reported by See et al. (2004) suggest that, in pigs, the response to ractopamine in the gilts, being phenotypically leaner, was an improvement in lean muscle growth, whereas the response of barrows to ractopamine was an increase in BW with an improvement in gain efficiency.

In cattle, the response to administration of ractopamine in steers has demonstrated consistent results with an average of 5 to 6 kg of HCW added due to ractopamine feeding (Gruber et al., 2007; Sachtleben et al., 2006b; Schroeder et al., 2005b). Response of heifers to ractopamine feeding has demonstrated on average an additional 0 to 5 kg of HCW due to ractopamine feeding (Laudert et al., 2007; Quinn et al., 2006; Schroeder et al., 2005d). These results are inconsistent due to a lack of response such as that reported by Quinn et al. (2006).

## **Conclusions**

In the context of this review,  $\beta$ -agonists are growth promotants that are used extensively to improve growth. With the FDA approval of two  $\beta$ -agonists, ractopamine and zilpaterol, utilization of these compounds in the United States may increase in the years to come. The addition of  $\beta$ -agonists to the diets of meat producing animals leads to improvements in daily gains and gain efficiencies as well as to increased kilograms of lean carcass produced, with the response being dependent on species, gender, and the  $\beta$ -agonist used. The process is accomplished due to the repartitioning effects of  $\beta$ -agonists on nutrient supplies. Furthermore, muscle fiber composition and/or diameter are altered in a way that permits skeletal muscle hypertrophy. Skeletal muscle hypertrophy results from changes in protein turnover rates. In general,  $\beta$ -agonists inhibit protein degradation, whereas protein synthesis is seldom affected.  $\beta$ -agonists appear not to affect DNA accretion; subsequently skeletal muscle satellite cell proliferation and differentiation are not affected. It is unclear whether IGF-I mediates any of the actions of  $\beta$ -agonists, and this warrants further investigation.

## References

- Aalhus, J. L., A. L. Schaefer, A. C. Murray, and S. D. M. Jones. 1992. The effect of ractopamine on myofiber distribution and morphology and their relation to meat quality in swine. *Meat Sci.* 31:397-409.
- Anderson, P. T. 1991. Trenbolone acetate as a growth promotant. *Com. Cont. Educ. Pract.* 13:1179-1190.
- Anderson, P. T., W. G. Helferich, L. C. Parkhill, R. A. Merkel, and W. G. Bergen. 1990. Ractopamine increases total and myofibrillar protein synthesis in cultured rat myotubes. *J. Nutr.* 120:1677-1683.
- Anderson, D. B., E. L. Veenhuizen, A. L. Schroeder, D. J. Jones, and D. L. Hancock. 1991. The use of phenethanolamines to reduce fat and increase leanness in meat animals. Page 43 in *Proc. Symp. Fat Cholesterol Reduced Foods – Adv. Appl. Biotechnol. Series. C.* Haberstroh and C. E. Morris, ed. Portfolio Publishing Company, New Orleans, LA.
- Apple, J. K., C. V. Maxwell, D. C. Brown, K. G. Friesen, R. E. Musser, Z. B. Johnson, and T. A. Armstrong. 2004. Effects of dietary lysine and energy density on performance and carcass characteristics of finishing pigs fed ractopamine. *J. Anim. Sci.* 82:3277-3287.
- Armstrong, T. A., D. J. Ivers, J. R. Wagner, D. B. Anderson, W. C. Weldon, and E. P. Berg. 2004. The effect of dietary ractopamine concentration and duration of feeding on growth performance, carcass characteristics, and meat quality of finishing pigs. *J. Anim. Sci.* 82:3245-3253.
- Attaix, D., E. Aourousseau, L. Combaret, A. Kee, D. Larbaud, C. Ralliere, B. Souweine, D. Taillandier, and T. Tilignac. 1998. Ubiquitin-proteosome dependent proteolysis in skeletal muscle. *Reprod. Nutr. Dev.* 38:153-165.
- Avendaño-Reyes, L., V. Torres-Rodríguez, F. J. Meraz-Murillo, C. Pérez-Linares, F. Figueroa-Saavedra, and P. H. Robinson. 2006. Effects of two  $\beta$ -adrenergic agonists on finishing performance, carcass characteristics, and meat quality of feedlot steers. *J. Anim. Sci.* 84:3259-3265.
- Awede, B. L., J. P. Thissen, and J. LeBacq. 2002. Role of IGF-I and IGF-BPs in the changes of mass and phenotype induced in rat soleus muscle by clenbuterol. *Am. J. Physiol. Endocrinol. Metab.* 282:E31-E37.

- Bark, L. J., T. S. Stahly, G. L. Cromwell, and J. Miyat. 1992. Influence of genetic capacity for lean tissue growth on rate and efficiency of tissue accretion in pigs fed ractopamine. *J. Anim. Sci.* 70:3391-3400.
- Baker, D. H., R. S. Katz, and R. A. Easter. 1975. Lysine requirements of growing pigs at two levels of dietary protein. *J. Anim. Sci.* 40:851-856.
- Bartle, S. J., R. L. Preston, R. E. Brown, and R. J. Grant. 1992. Trenbolone acetate/estradiol combinations in feedlot steers: Dose-response and implant carrier effects. *J. Anim. Sci.* 70:1326-1332.
- Beermann, D. H., W. R. Butler, D. E. Hogue, V. K. Fishell, R. H. Dalrymple, C. A. Ricks, and C. G. Scanes. 1987. Cimaterol-induced muscle hypertrophy and altered endocrine status in lambs. *J. Anim. Sci.* 65:1514-1524.
- Beermann, D. H., D. E. Hogue, V. K. Fishell, R. H. Dalrymple, and C. A. Ricks. 1986. Effects of cimaterol and fishmeal on performance, carcass characteristics and skeletal muscle growth in lambs. *J. Anim. Sci.* 62:370-380.
- Benson, D. W., T. Foley-Nelson, W. T. Chance, F. S. Zhang, J. H. James, and J. E. Fischer. 1991. Decreased myofibrillar protein breakdown following treatment with clenbuterol. *J. Surg. Res.* 50:1-5.
- Bergen, W. G., S. E. Johnson, D. M. Skjaerlund, A. S. Babiker, N. K. Ames, R. A. Merkel, and D. B. Anderson. 1989. Muscle protein metabolism in finishing pigs fed ractopamine. *J. Anim. Sci.* 67:2255-2262.
- Bohorov, O., P. J. Buttery, J. H. R. D. Corriea, and J. B. Soar. 1987. The effect of the  $\beta$ -2-adrenergic agonist clenbuterol or implantation with oestradiol plus trenbolone acetate on protein metabolism in wether lambs. *Br. J. Nutr.* 57:99-107.
- Bouffault, J. C., and J. P. Willemart. 1983. Anabolic activity of trenbolone acetate alone or in association with estrogens Pages 155 – 192 in *Anabolics in Animal Production*. E. Meissonnier, ed., Office International des Epizootics, Paris, France.
- Bowen, W. P., D. J. Flint, and R. G. Vernon. 1992. Regional and interspecific differences in the ligand binding properties of  $\beta$ -adrenergic receptors of individual white adipose tissue depots in the sheep and rat. *Biochem. Pharmacol.* 44:681-686.
- Byrem, T. M., D. H. Beermann, and T. F. Robinson. 1998. The beta-agonist cimaterol directly enhances chronic protein accretion in skeletal muscle. *J. Anim. Sci.* 76:988-998.

- Carter, S. D., G. L. Cromwell, M. D. Lindermann, L. W. Turner, and T. C. Bridges. 1996. Reducing N and P excretion by dietary manipulation in growing and finishing pigs. *J. Anim. Sci.* 74 (Suppl.1):59. (Abstr.)
- Chikuni, K., S. Muroya, and I. Nakajima. 2004. Myosin heavy chain isoforms expressed in bovine skeletal muscles. *Meat Sci.* 67:87-94.
- Claeys, M. C., D. R. Mulvaney, F. D. McCarthy, M. T. Gore, D. N. Marple, and J. L. Sartin. 1989. Skeletal muscle protein synthesis and growth hormone secretions in young lambs treated with clenbuterol. *J. Anim. Sci.* 67:2245-2254.
- Colins, S., M. Bouvier, M. A. Bolanowski, M. G. Caron, and R. J. Lefkowitz. 1989. cAMP stimulates transcription of the  $\beta_2$ -adrenergic receptor gene in response to short-term agonist exposure. *Proc. Natl. Acad. Sci. USA* 86:4853-4857.
- Cromwell, G. L., J. D. Kemp, T. S. Stahly, and R. H. Dalrymple. 1988. Effects of dietary level and withdrawal time on the efficacy of cimaterol as a growth repartitioning agent in finishing swine. *J. Anim. Sci.* 66:2193-2199.
- Dalrymple, R. H., P. K. Baker, P. E. Gingher, D. L. Ingle, J. M. Pensack, and C. A. Ricks. 1984. A repartitioning agent to improve performance and carcass composition of broilers. *Poult. Sci.* 63:2376-2383.
- Dawson, J. M., J. Craigon, P. J. Buttery, and D. E. Beever. 1993. Influence of diet and  $\beta$ -agonist administration on plasma concentrations of growth hormone and insulin-like growth factor-I in young steers. *Br. J. Nutr.* 70:93-102.
- Depreux, F. F. S., A. L. Grant, D. B. Anderson, and D. E. Gerrard. 2002. Paylean alters myosin heavy chain isoform content in pig muscle. *J. Anim. Sci.* 80:1888-1894.
- Dumont, J. E., J. C. Jauniaux, and P. P. Roger. 1989. The cyclic AMP-mediated stimulation of cell proliferation. *Trends Biochem. Sci.* 14:67-71.
- Dunn, J. D., B. J. Johnson, J. P. Kayser, A. T. Waylan, E. K. Sissom, and J. S. Drouillard. 2003. Effect of flax supplementation and a combined trenbolone acetate and estradiol implant on circulating insulin-like growth factor-I and muscle insulin-like growth factor-I messenger RNA levels in beef cattle. *J. Anim. Sci.* 81:3028-3034.
- Dunshea, F. R., and R. H. King. 1994. Temporal response of plasma metabolites to ractopamine treatment in the growing pig. *Aust. J. Agric. Res.* 45:1683-1692.



- Easter, R. A., and D. H. Baker. 1980. Lysine and protein levels in corn-soybean meal diets for growing-finishing swine. *J. Anim. Sci.* 50:467-471.
- Eisemann, J. H., and D. G. Bistol. 1998. Change in insulin sensitivity or responsiveness is not a major component of the mechanism of action of ractopamine in beef steers. *J. Nutr.* 128:505-511.
- Forsberg, N. E., M. A. Ilian, A. Ali-Bar, P. R. Cheeke, and N. B. Wehr. 1989. Effects of cimaterol on rabbit growth and myofibrillar protein degradation and on calcium-dependent proteinase and calpastatin activities in skeletal muscle. *J. Anim. Sci.* 67:3313-3321.
- Friend, D. W., and T. M. MacIntyre. 1970. Paired feeding and metabolism trials comparing barrows and gilts. *J. Anim. Sci.* 30:931-934.
- Goll D. E., V. F. Thompson, H. Li, W. Wei, and J. Cong. 2002. The calpain system. *Physiol. Rev.* 83:731-801.
- Goll D. E., V. F. Thompson, R. G. Taylor, and A. Ouali. 1998. The calpain system and skeletal muscle growth. *Can. J. Anim. Sci.* 78:503-512.
- Gonzales, J. M., J. N. Carter, D. D. Johnson, S. E. Ouellette, and S. E. Johnson. 2007. Effect of ractopamine-hydrochloride and trenbolone acetate on longissimus muscle fiber area, diameter, and satellite cell numbers in cull beef cows. *J. Anim. Sci.* 85:1893-1901.
- Grant, A. L., W. G. Helferich, R. A. Merkel, and W. G. Bergen. 1990. Effects of phenethanolamines and propranolol on the proliferation of cultured chick breast muscle satellite cells. *J. Anim. Sci.* 68:652-658.
- Grant, A. L., D. M. Skjaerlund, W. G. Helferich, W. G. Bergen, and R. A. Merkel. 1993. Skeletal muscle growth and expression of skeletal muscle  $\alpha$ -actin mRNA and insulin-like growth factor I mRNA in pigs during feeding and withdrawal of ractopamine. *J. Anim. Sci.* 71:3319-3326.
- Gruber, S. L., J. D. Tatum, T. E. Engle, M. A. Mitchell, S. B. Laudert, A. L. Schroeder, and W. J. Platter. 2007. Effects of ractopamine supplementation on growth performance and carcass characteristics of feedlot steers differing in biological type. *J. Anim. Sci.* 85:1809-1815.

- Gunawan, A. M., G. T. Richert, A. P. Schinckel, A. L. Grant, and D. E. Gerrard. 2007. Ractopamine induces differential gene expression in porcine skeletal muscle. *J. Anim. Sci.* 85:2115-2124.
- Gwartney, B. L., S. J. Jones, and C. R. Calkins. 1992. Response time of broiler chickens to cimaterol: meat tenderness, muscle composition, fiber size, and carcass characteristics. *J. Anim. Sci.* 70:2144-250.
- Hausdorff, W. P., M. G. Caron, and R. J. Lefkowitz. 1990. Turing off the signal; desensitization of  $\beta$ -adrenergic receptor function. *FASEB J.* 4:2881-2889.
- Hawke, T. J., and D. J. Garry. 2001. Myogenic satellite cells: physiology to molecular biology. *J. Appl. Physiol.* 91:534-551.
- Hayden, J. M., W. G. Bergen, and R. A. Merkel. 1992. Skeletal muscle protein metabolism and serum growth hormone, insulin, and cortisol concentrations in growing steers implanted with estradiol 17 $\beta$ , trenbolone acetate, or estradiol 17 $\beta$  plus trenbolone acetate. *J. Anim. Sci.* 70:2109-2119.
- Helferich, W. G., D. B. Jump, D. B. Anderson, D. M. Skjaerlund, R. A. Merkel, and W. G. Bergen. 1990. Skeletal muscle  $\alpha$ -actin synthesis is increased pretranslationally in pigs fed the phenethanolamine ractopamine. *Endocrinol.* 126:3096-3100.
- Hollis, G. R., and S. E. Curtis. 2001. General characteristics of the U.S. swine industry. Pages 19-30 in *Swine Nutrition*. 2nd ed. A. J. Lewis and L. L. Southern, ed. CRC Press, Boca Raton, FL.
- January, B., A. Seibold, B. Whaley, R. W. Hipkin, D. Lin, A. Schonbrunn, R. Barber, and R. B. Clark. 1997.  $\beta$ 2-Adrenergic receptor desensitization, internalization, and phosphorylation in response to full and partial agonists. *J. Biol. Chem.* 272:23871-23879.
- Johnson, B. J., P. T. Anderson, J. C. Meiske, and W. R. Dayton. 1996a. Effects of a combined trenbolone acetate and estradiol implant on feedlot performance, carcass characteristics, and carcass composition of feedlot steers. *J. Anim. Sci.* 74:363-371.
- Johnson, B. J., N. Halstead, M. E. White, M. R. Hathaway, A. DiCostanzo, and W. R. Dayton. 1998b. Activation state of muscle satellite cells isolated from steers implanted with a combined trenbolone acetate and estradiol implant. *J. Anim. Sci.* 76:2779-2786.
- Johnson, B. J., M. R. Hathaway, P. T. Anderson, J. C. Meiske, and W. R. Dayton. 1996b.

- Stimulation of circulating insulin-like growth factor I (IGF-I) and insulin-like growth factor binding proteins (IGFBP) due to administration of a combined trenbolone acetate and estradiol implant in feedlot cattle. *J. Anim. Sci.* 74:372-379.
- Johnson, B. J., M. E. White, M. R. Hathaway, C. J. Christians, and W. R. Dayton. 1998a. Effect of a combined trenbolone acetate and estradiol implant on steady-state IGF-I mRNA concentrations in the liver of wethers and the longissimus muscle of steers. *J. Anim. Sci.* 76:491-497.
- Johnson, G. L., B. B. Wolfe, T. K. Harden, P. B. Molinoff, and J. P. Perkins. 1978. Role of  $\beta$ -adrenergic receptors in catecholamine-induced desensitization of adenylate cyclase in human astrocytoma cells. *J. Biol. Chem.* 253:1472-1480.
- Jones, R. W., R. A. Easter, F. K. McKeith, R. H. Dalrymple, H. M. Maddock, and P. J. Bechtel. 1985. Effect of the  $\beta$ -adrenergic agonist cimaterol (CL 263,780) on the growth and carcass characteristics of finishing swine. *J. Anim. Sci.* 61:905-913.
- Kamanga-Sollo, E., M. S. Pampusch, M. E. White, M. R. Hathaway, and W. R. Dayton. 2005. Insulin-like growth factor binding protein (IGFBP)-3 and IGFBP-5 mediate TGF- $\beta$ - and myostatin-induced suppression of proliferation in porcine embryonic myogenic cell cultures. *Exp. Cell Res.* 311:167-176.
- Kim, Y. S., Y. B. Lee, and R. H. Dalrymple. 1987. Effect of repartitioning agent cimaterol on growth, carcass and skeletal muscle characteristics in lambs. *J. Anim. Sci.* 65:1392-1399.
- Kim, Y. S., R. D. Sainz, R. J. Summers, and P. Molenaar. 1992. Cimaterol reduces  $\beta$ -adrenergic receptor density in rat skeletal muscles. *J. Anim. Sci.* 70:115-122.
- Kopin, I. J. 1985. Catecholamine metabolism: Basic aspects and clinical significance. *Pharmacol. Rev.* 37:333-364.
- Laudert, S. B., G. J. Vogel, A. L. Schroeder, and W. J. Platter. 2007. Effect of ractopamine HCl on growth and carcass traits of finishing heifers fed to slaughter. *J. Anim. Sci.* 85 (Suppl. 1): 663. (Abstr.)
- Liggett, S. B., and J. R. Raymond. 1993. Pharmacology and molecular biology of adrenergic receptors. *Baillieres Clin. Endocrinol. Metab.* 7:279-305.
- Mauro, A. 1961. Satellite cells of skeletal muscle fibers. *J. Biochem. Biophys.* 9:493-495.
- McElligott, M. A., and L. Y. Chung. 1988. Protein turnover and growth of L8 muscle cultures in serum from rats fed clenbuterol. *Growth Dev. Aging* 52:185-191.

- McNeel, R. L., and H. J. Mersmann. 1999. Distribution and quantification of beta<sub>1</sub>-, beta<sub>2</sub>-, and beta<sub>3</sub>-adrenergic receptor subtype transcripts in porcine tissues. *J. Anim. Sci.* 77:611-621.
- Mersmann, H. J. 1998. Overview of the effects of  $\beta$ -adrenergic receptor agonists on animal growth including mechanisms of action. *J. Anim. Sci.* 76:160-172.
- Mill, S., and H. J. Mersmann. 1995. Beta-adrenergic agonists, their receptors, and growth: special reference to the peculiarities in pigs. Page 1 of *The Biology of Fat in Meat Animals: Current Advances*. S. B. Smith and D. R. Smith, ed. American Society of Animal Science, Champaign, IL.
- Miller, M. F., D. K. Garcia, M. E. Coleman, P. A. Ekeren, D. K. Lunt, K. A. Wagner, M. Procknor, T. H. Welsh, Jr., and S. B. Smith. 1988. Adipose tissue, longissimus muscle and anterior pituitary growth and function in clenbuterol-fed heifers. *J. Anim. Sci.* 66:12-20.
- Moloney, A. P., P. Allen, D. B. Ross, G. Olson, and E. M. Convey. 1990. Growth, feed efficiency and carcass composition of finishing Friesian steers fed the  $\beta$ -adrenergic agonist L-644,969. *J. Anim. Sci.* 68:1269-1277.
- Moody, D. E., D. L. Hancock, and D. B. Anderson. 2000. Phenethanolamine repartitioning agents. Page 65 in *Farm Animal Metabolism and Nutrition*. J. P. F. D'Mello, ed. CABI Publishing, Wallingford, UK.
- Morgan, D. J. 1990. Clinical pharmacokinetics of  $\beta$ -agonists. *Clin. Pharmacokinet.* 18:270-294.
- Moss, F. P., and C. P. LeBlond. 1971. Satellite cells as the source of nuclei in muscle of growing rats. *Anat. Rec.* 170:421-435.
- O'Connor, R. M., W. R. Butler, K. D. Finnerty, D. E. Hogue, and D. H. Beermann. 1991. Acute and chronic hormone and metabolite changes in lambs fed the beta-agonist, cimaterol. *Dom. Anim. Endocrinol.* 8:537-548.
- O'Connor, R. M., W. R. Butler, D. E. Hogue, and D. H. Beermann. 1991. Temporal pattern of skeletal muscle changes in lambs fed cimaterol. *Dom. Anim. Endocrinol.* 8:549-554.
- Oksbjerg, N., P. Henckel, and T. Rolph. 1994. Effects of salbutamol, a  $\beta_2$ -adrenergic agonist, on muscles of growing pigs fed different levels of dietary protein. *Acta. Agric. Scand. Sect. A. Anim. Sci.* 44:12-19.

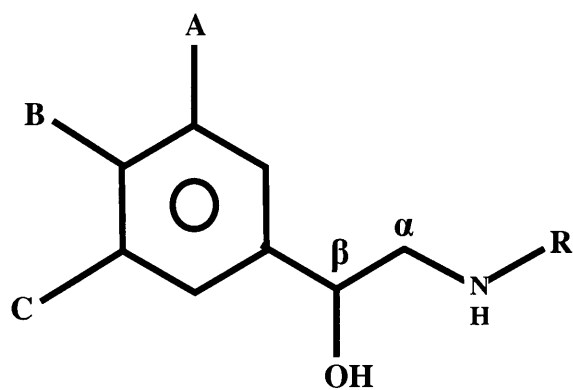
- Pampusch, M. S., B. J. Johnson, M. E. White, M. R. Hathaway, J. D. Dunn, A. T. Waylan, and D. R. Dayton. 2003. Time course of changes in growth factor mRNA levels in muscle of steroid-implanted and nonimplanted steers. *J. Anim. Sci.* 81:2733-2740.
- Parr, T., R. G. Bardsley, R. S. Gilmour, and P. J. Buttery. 1992. Changes in calpain and calpastatin mRNA induced by  $\beta$ -adrenergic stimulation of bovine skeletal muscle. *Eur. J. Biochem.* 208:333-339.
- Pette, D., and R. S. Staron. 1990. Cellular and molecular diversities of mammalian skeletal muscle fibers. *Rev. Physiol. Biochem. Pharmacol.* 116:1-76.
- Pietri-Rouxel, F., and A. D. Strosberg. 1995. Pharmacological characteristics and species-related variations of  $\beta_3$ -adrenergic receptors. *Fundam. Clin. Pharmacol.* 9:211-218.
- Plascencia, A., N. Torrentera, and R. A. Zinn. 1999. Influence of the  $\beta$ -agonist, zilpaterol, on growth performance and carcass characteristics of feedlot steers. *Proc. West. Sect. Am. Soc. Anim. Sci.* 50:331-334.
- Quinn, M. J., J. S. Drouillard, C. D. Reinhardt, A. S. Webb, J. M. Pozuelo, M. L. May, C. E. Walker, and S. J. Winterholler. 2006. The effects of dose and duration of ractopamine-HCl administration on finishing performance and carcass traits of non-implanted beef heifers. *J. Anim. Sci.* 84(Suppl. 1):120. (Abstr.)
- Reeds, P. J., S. M. Hay, P. M. Dorwood, and R. M. Palmer. 1986. Stimulation of muscle growth by clenbuterol: lack of effect on muscle protein biosynthesis. *Br. J. Nutr.* 56:249-258.
- Rehfeldt, C., R. Schadereit, R. Weikard, and K. Reichel. 1997. Effect of clenbuterol on growth, carcass and skeletal muscle characteristics in broiler chickens. *Br. Poult. Sci.* 38:366-373.
- Ricks, C. A., R. H. Dalrymple, P. K. Baker, and D. L. Ingle. 1984. Use of a  $\beta$ -agonists to alter fat and muscle deposition in steers. *J. Anim. Sci.* 59:1247-1255.
- Ruffolo, R. R. 1991. Chirality in  $\alpha$ - and  $\beta$ -adrenoceptor agonists and antagonists. *Tetrahedron* 47:9953-9980.
- Sachtleben, S., E. Thomas, W. Platter, and A. Schroeder. 2006a. Evaluation of feeding ractopamine (Optaflexx<sup>®</sup>) with various levels of dietary crude protein on growth performance in feedlot steers. *J. Anim. Sci.* 84(Suppl. 1):118. (Abstr.)
- Sachtleben, S., E. Thomas, W. Platter, and A. Schroeder. 2006b. Evaluation of feeding ractopamine (Optaflexx<sup>®</sup>) with various levels of dietary crude protein on carcass characteristics in feedlot steers. *J. Anim. Sci.* 84(Suppl. 1):119. (Abstr.)

- Sainz, R. D., Y. S. Kim, F. R. Dunshea, and R. G. Campbell. 1993. Effects of ractopamine in pig muscles: histology, calpains and  $\beta$ -adrenergic receptors. *Aust. J. Agric. Res.* 44:1441-1448.
- Sainz, R. D., J. E. Wolff, and P. M. Dobbie. 1990. Cimaterol reduces the responses of rat muscles to insulin and IGF-I. *Aust. J. Agric. Res.* 41:733-740.
- Schiavetta, A. M., M. F. Miller, D. K. Lunt, S. K. Davis, and S. B. Smith. 1990. Adipose tissue cellularity and muscle growth in young steers fed the  $\beta$ -adrenergic agonist clenbuterol for 50 days and after 78 days of withdrawal. *J. Anim. Sci.* 68:3614-3523.
- Schroeder, A., D. Hancock, D. Mowrey, S. Laudert, G. Vogel, and D. Polser. 2005a. Dose titration of Optaflexx<sup>®</sup> (ractopamine-HCl) evaluating the effects on growth performance in feedlot steers. *J. Anim. Sci.* 83(Suppl. 1):163. (Abstr.)
- Schroeder, A., D. Hancock, D. Mowrey, S. Laudert, G. Vogel, and D. Polser. 2005b. Dose titration of Optaflexx<sup>®</sup> (ractopamine-HCl) evaluating the effects on standard carcass characteristics in feedlot steers. *J. Anim. Sci.* 83(Suppl. 1):164. (Abstr.)
- Schroeder, A., D. Hancock, D. Mowrey, S. Laudert, G. Vogel, and D. Polser. 2005c. Dose titration of Optaflexx<sup>®</sup> (ractopamine-HCl) evaluating the effects on growth performance in feedlot heifers. *J. Anim. Sci.* 83(Suppl. 1):170. (Abstr.)
- Schroeder, A., D. Hancock, D. Mowrey, S. Laudert, G. Vogel, and D. Polser. 2005d. Dose titration of Optaflexx<sup>®</sup> (ractopamine-HCl) evaluating the effects on standard carcass characteristics in feedlot heifers. *J. Anim. Sci.* 83(Suppl. 1):171. (Abstr.)
- See, M. T., T. A. Armstrong, and W. C. Weldon. 2004. Effect of ractopamine feeding program on growth performance and carcass composition in finishing pigs. *J. Anim. Sci.* 82:2474-2480.
- Sibley, D. R., J. L. Benovic, M. G. Caron, and R. J. Lefkowitz. 1987. Regulation of transmembrane signaling by receptor phosphorylation. *Cell* 48:913-922.
- Sillence, M. N., and M. L. Matthews. 1994. Classical and atypical binding sites for  $\beta$ -adrenoreceptor ligands and activation of adenylyl cyclase in bovine skeletal muscle and adipose tissue membranes. *Br. J. Pharmacol.* 111:866-872.
- Sissom, E. K., C. D. Reinhardt, J. P. Hutcheson, W. T. Nichols, D. A. Yates, R. S. Swingle, and B. J. Johnson. 2007. Response to ractopamine-HCl in heifers is altered by implant strategy across days on feed. *J. Anim. Sci.* 85:2125-2132.

- Smith, D. J. 1998. The pharmacokinetics, metabolism, and tissue residues of  $\beta$ -adrenergic agonists in livestock. *J. Anim. Sci.* 76:173-194.
- Smith, S. B., D. K. Garcia, and D. B. Anderson. 1989. Elevation of specific mRNA of longissimus muscle of steers fed ractopamine. *J. Anim. Sci.* 67:3495-3502.
- Smith, D. J., and G. D. Paulson. 1997. Distribution, elimination, and residues of [ $^{14}\text{C}$ ] clenbuterol HCl in Holstein calves. *J. Anim. Sci.* 75:454-461.
- Sosnicki, A. 1987. Association of micrometric traits on meat quality, fattening and slaughter traits in the pig. *J. Anim. Sci.* 64:1412-1418.
- Spurlock, M. E., J. C. Cusumano, S. Q. Ji, D. B. Anderson, C. K. Smith II, D. L. Hancock, and S. E. Mills. 1994. The effect of ractopamine on  $\beta$ -adrenoreceptor density and affinity in porcine adipose and skeletal muscle tissue. *J. Anim. Sci.* 72:75-80.
- Van Liefde, I., A. Van Ermen, A. Van Witzenburg, N. Fraeyman, and G. Vauquelin. 1994. Species and strain-related differences in the expression and functionality of  $\beta$ -adrenoceptor subtypes in adipose tissue. *Arch. Int. Pharmacodyn.* 327:69-86.
- Vestergaard, M., P. Henckel, N. Oksbjerg, and K. Sejrsen. 1994. The effect of cimaterol on muscle characteristics, capillary supply, and metabolic potentials of longissimus and semitendinosus muscles of young Friesian bulls. *J. Anim. Sci.* 72:2298-2306.
- Webster, M. J., R. D. Goodband, M. D. Tokach, J. A. Unruh, J. L. Nelssen, S. S. Dritz, D. E. Real, J. M. DeRouchey, J. C. Woodworth, and T. A. Marsteller. 2002. Interactive effects between Paylean<sup>®</sup> (ractopamine HCl) and dietary lysine on finishing pig growth performance, carcass characteristics and tissue accretion. *J. Anim. Sci.* 80(Suppl.2):78. (Abstr.)
- Weiner, N. 1980. Norepinephrine, epinephrine, and the sympathomimetic amines. Page 138 in *The Pharmacological Basis of Therapeutics* (6<sup>th</sup> Ed.). A. Goodman-Gilman, L. S. Goodman, A. Gilman, S. E. Mayer, and K. L. Melmon, ed. Macmillan Publishing Co., New York, NY.
- Wellenreiter, R. H., and L. V. Tonkinson. 1990. Effect of ractopamine hydrochloride on growth performance of turkeys. *Poult. Sci.* 69(Suppl. 1):142. (Abstr.)
- Wheeler, T. L., and M. Koohmaraie. 1992. Effects of  $\beta$ -adrenergic agonist L<sub>644,969</sub> on muscle protein turnover, endogenous proteinase activities, and meat tenderness in steers. *J. Anim. Sci.* 70:3035-3043.

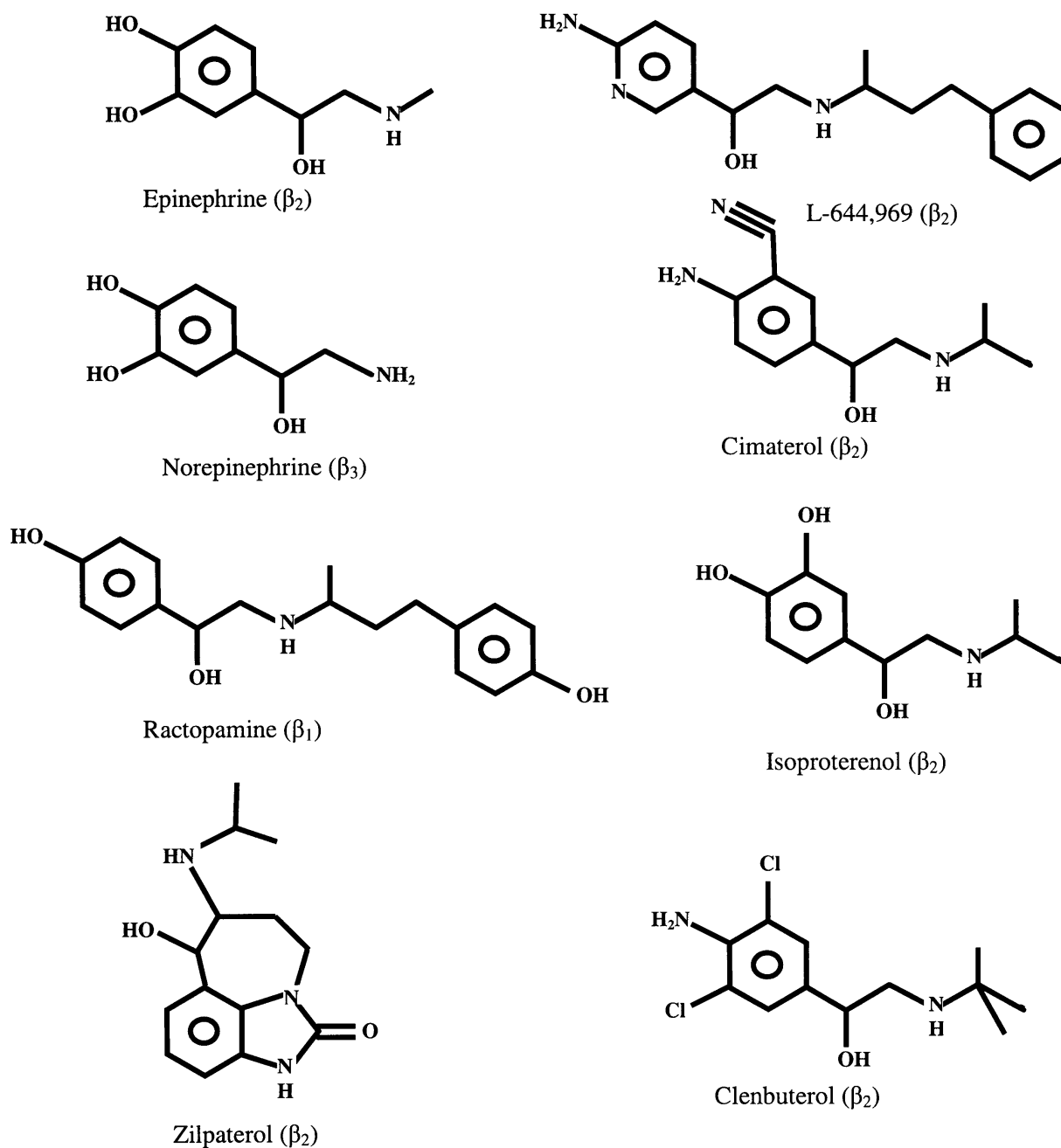
- White, M. E., B. J. Johnson, M. R. Hathaway, and W. R. Dayton. 2003. Growth factor messenger RNA levels in muscle and liver of steroid-implanted and nonimplanted steers. *J. Anim. Sci.* 81:965-972.
- Winterholler, S. J. 2006. Effect of ractopamine-HCl and steroid implantation on yearling steer feedlot performance, carcass characteristics, and skeletal muscle gene expression. M. S. Thesis, Kansas State University, Manhattan.
- Winterholler, S. J., G. L. Parsons, C. D. Reinhardt, J. P. Hutcheson, W. T. Nichols, D. A. Yates, R. S. Swingle, and B. J. Johnson. 2007. Response to ractopamine-hydrogen chloride is similar in yearling steers across days on feed. *J. Anim. Sci.* 85:413-419.
- Witkamp, R. F. 1996. Pharmacological and toxicological properties of  $\beta$ -adrenergic agonists. Page 113 in *Residues of Veterinary Drugs and Mycotoxins in Animal Products: New Methods for Risk Assessment and Quality Control*, G. Enne, H. A. Kuiper, and A. Valentini, ed. Wageningen Pers, Wageningen, The Netherlands.
- Yimlamai, T., S. L. Dodd, S. E. Borst, and S. Park. 2005. Clenbuterol induces muscle-specific attenuation of atrophy through effects on the ubiquitin-proteasome pathway. *J. Appl. Physiol.* 99:71-80.
- Young, R. B., K. Y. Bridge, A. J. Wiethrich, and D. L. Hancock. 2002. Effect of serum from chickens treated with clenbuterol on myosin accumulation,  $\beta$ -adrenergic receptor population, and cyclic AMP synthesis in embryonic chicken skeletal muscle cell cultures. *In Vitro Cell. Dev. Biol.-Anim.* 38:102-110.
- Young, R. B., D. M. Moriarity, C. E. McGee, W. R. Farrar, and H. E. Richter. 1990. Protein metabolism in chicken muscle cell cultures treated with cimaterol. *J. Anim. Sci.* 68:1158-1169.
- Young, O. A., S. Watkins, J. M. Oldham, and J. J. Bass. 1995. The role of insulin-like growth factor I in clenbuterol-stimulated growth in growing lambs. *J. Anim. Sci.* 73:3069-3077.
- Zammit, P. S., and J. R. Beauchamp. 2001. The skeletal muscle satellite cell: stem cell or son of stem cell? *Differentiation* 68:193-204.





**Figure 1.1 Phenethanolamine backbone structure**

Adapted from Smith (1998)



**Figure 1.2 Names, structures, and classification of some phenethanolamines.**

Adapted from Moody et al. (2000), Mersmann et al. (1998), and Smith (1998)

**CHAPTER 2 - The Effects of Ractopamine and Protein Source on  
Growth Performance and Carcass Characteristic in Finishing  
Heifers**

D. K. Walker, E. C. Titgemeyer, J. S. Drouillard, E. R. Loe, B. E. Dejenbusch, and A. S.

Webb

Used by permission of the Journal of Animal Science, 2006 84:2795-2800.

## Abstract

An experiment was conducted to determine the relationship between feeding ractopamine and different amounts of MP on growth and carcass characteristics of feedlot heifers. Seventy-two crossbred heifers (475 kg initial BW) were fed individually a diet based on steam-flaked corn for ad libitum intake for 29 d. Heifers were implanted with 140 mg trenbolone acetate and 14 mg estradiol-17 $\beta$  60 d before the experiment. Treatments were arranged as a 2  $\times$  3 factorial and included 0 or 200 mg ractopamine-HCl (23 ppm)/d, and either urea, solvent soybean meal (SSBM), or expeller soybean meal (ESBM) as the predominant protein supplement. The amounts of MP supplied by the urea, SSBM, and ESBM diets were 688, 761, and 808 g/d, respectively, calculated according to Level 1 of the NRC (2000) model. Body weights were obtained 1 d before ractopamine feeding and at slaughter. Blood samples were obtained 1 d before starting the experiment and 13 d later. Ractopamine improved ADG, gain efficiency, carcass-adjusted ADG, and carcass-adjusted gain efficiency ( $P < 0.01$ ). For ADG, heifers demonstrated a ractopamine  $\times$  protein source interaction ( $P < 0.05$ ); heifers not fed ractopamine had greater ADG when fed ESBM than when fed urea, whereas for heifers fed ractopamine there were no differences ( $P \geq 0.10$ ) among protein supplements. This interaction was not observed for carcass-adjusted ADG ( $P = 0.60$ ). Final live weights ( $P = 0.02$ ) and carcass weights ( $P = 0.01$ ) were greater with ractopamine feeding. Carcass marbling scores and yield grades were not affected by ractopamine or protein source ( $P \geq 0.39$ ). Plasma total  $\alpha$ -amino nitrogen and glucose concentrations decreased more from pretreatment concentrations when heifers were fed ractopamine ( $P < 0.05$ ). Feeding ractopamine to heifers for 28 d before slaughter improved daily ADG and efficiency of gain without any large effects on carcass characteristics. The MP supply does not need to be increased from that provided by finishing diets based on steam-flaked corn with urea as the primary N supplement to allow maximal response to ractopamine by finishing heifers.

## **Introduction**

Diets for feedlot cattle typically contain 12 to 14% CP, with a majority of the protein as degradable intake protein (DIP; Galyean, 1996). Milton et al. (1997) showed that addition of undegradable intake protein (UIP) to a diet based on dry-rolled corn, which increased CP from 10.6 to 12.6%, did not improve performance of finishing steers and that DIP derived from corn and urea was sufficient to maximize performance. Furthermore, increasing CP in a diet based on steam-flaked corn from 13% to 14.5% had no effect on feedlot performance, and urea was more effective in improving efficiency than was cottonseed meal (Gleghorn et al., 2004). Taken from these data, feedlot diets containing urea as the predominant protein supplement supply adequate amounts of DIP and are sufficient to optimize gain and efficiency of feedlot cattle.

Requirements for MP differ depending on rate and composition of gain. Thus, MP requirements could be increased by growth promotants that cause cattle to grow faster and deposit more lean tissue. Ractopamine is a  $\beta_1$  adrenergic agonist recently introduced for use in cattle in the United States. Feeding  $\beta$  agonists (clenbuterol, cimaterol, zilpaterol) to cattle leads to marked alterations in metabolism that increase leanness and muscle accretion, with only moderate effects on fat deposition (Mersmann, 1998), which could increase MP requirements. Alternately,  $\beta$  agonists could enhance the efficiency with which cattle use MP, thereby leading to no change or even a decrease in MP requirements. Feeding ractopamine to pigs increased lean tissue deposition, but pigs fed ractopamine required no change in dietary protein to achieve optimal response to ractopamine (Mitchell et al., 1991; Xiao et al., 1999). Our objectives were to determine the impact of feeding ractopamine to heifers for 28 d before slaughter, and to determine whether the response of heifers to ractopamine is influenced by supplemental protein sources supplying differing amounts of MP.

## **Materials and Methods**

Procedures for this study were approved by the Kansas State University Institutional Animal Care and Use Committee.

Mixed breed, medium- to large-framed heifers that were predominantly continental crosses ( $n = 72$ ; 475 kg initial BW, range 393 to 583 kg) were used in a randomized complete block design with a  $2 \times 3$  factorial arrangement of treatments to evaluate the effects of

ractopamine, and supplemental protein source (supplying differing amounts of MP) on growth and carcass characteristics. Dietary treatments (Table 2.1) consisted of finishing diets based on steam-flaked corn, formulated to contain 13.7% CP, with urea, solvent soybean meal (SSBM), or expeller soybean meal (ESBM) as the primary supplemental protein source and with 0 or 200 mg/d ractopamine-HCl (23 ppm; provided as Optaflexx; Elanco Animal Health, Indianapolis, IN). Heifers were implanted with 140 mg trenbolone acetate and 14 mg estradiol-17 $\beta$  (Revalor H; Intervet, Millsboro, DE) 60 d before the experiment started. All heifers had been fed and housed individually for 49 d prior to initiation of our trial, and all were fed a common diet based on steam-flaked corn for ad libitum consumption for 27 d prior to initiation of our trial. Dietary treatments, which had little impact on heifer performance, had been applied between 27 and 49 d before initiation of our trial, and these prior treatments were randomly distributed among our treatments. Heifers were allotted to one of 12 blocks based on frame size (score of 1 to 3, each representing roughly one-third of the heifers as the largest, middle, and smallest frame sizes), body condition (2 groups, with 1 containing the thinnest one-third of the heifers and the other containing the remainder), and BW, and were housed in individual pens (1.5  $\times$  6 m). Heifers were fed individually and were given ad libitum access to their diet for 28 d, which represents both the shortest feeding time for which ractopamine usage is labeled as well as the most typical feeding period for ractopamine by the feedlot industry. Heifers were fed once daily at 0900 with bunk management designed to yield slick bunks at feeding. Initial BW were measured 1 d before initiation of ractopamine feeding at 0800, and final BW were obtained at 0500 directly before heifers were shipped to a commercial abattoir.

Hot carcasses were weighed at slaughter. Percentage of KPH, 12th-rib fat thickness, marbling score, LM area, USDA yield grade (calculated), and marbling score of the carcasses were measured after a 24-h chill. Marbling scores were determined by a USDA grader.

Jugular blood samples were collected 1 d before initiation of ractopamine feeding at 0800 and 13 d later at 0800. Blood was collected into vacuum tubes (Becton Dickinson, Franklin Lakes, NJ) containing sodium heparin, immediately placed on ice, and centrifuged for 20 min at 1,000  $\times$  g to obtain plasma. Plasma samples were stored ( $-20^{\circ}\text{C}$ ) for later analysis of plasma urea (Marsh et al., 1965), total  $\alpha$ -amino nitrogen (Palmer and Peters, 1969), glucose, and lactate (glucose/lactate auto-analyzer; YSI 2300 STAT Plus; YSI Inc., Yellow Springs, OH).

Data were analyzed by using the MIXED procedure of SAS System for Windows Release 8.1 (SAS Inst. Inc., Cary, NC). The model included the effects of protein source, ractopamine, and ractopamine  $\times$  protein. Block was included as a random effect. For final BW and HCW, initial BW was included as a covariate. Treatment means were computed with the LSMEANS option. When an *F*-test for protein or for the ractopamine  $\times$  protein interaction was significant ( $P < 0.05$ ), individual treatment means were separated with pairwise *t*-tests among all means.

Diets were evaluated with Level 1 of the NRC (2000) model and with the Cornell Net Carbohydrate and Protein System 5.0 (CNCPS; Fox et al., 1992; Russell et al., 1992; Sniffen et al., 1992). Inputs included DM intakes of 8.7 kg/d and BW of 480 kg (averages for our experiment). For the NRC (1996-) evaluation, ESBM was assumed to contain 48% CP, with 45% of CP being DIP; SSBM was assumed to contain 55% CP, with 65% of CP being DIP. For both models, concentrated separator byproduct was considered similar to beet molasses with 20% ash and 17.6% CP, with 100% of CP being DIP. Nutrient contents of other feedstuffs were as specified by the models' databases.

## Results and Discussion

Performance data for heifers are presented in Table 2.2. Dry matter intake was not affected by ractopamine or protein source ( $P \geq 0.13$ ). Average daily gain was increased 18% by ractopamine ( $P = 0.02$ ). When daily gains were calculated from carcass weights, ractopamine increased ADG by 25% ( $P = 0.008$ ). Basing daily gains on carcass weights removes potential differences in gut fill, perhaps allowing a more accurate evaluation of heifer growth. Similarly, Carroll et al. (1990) demonstrated an 11% increase in ADG in response to feeding 20 ppm ractopamine for 38 to 45 d in feedlot steers. Preston et al. (1990) reported a 25% increase in ADG when feeding 20 ppm ractopamine to finishing steers for 46 d.

Ractopamine increased ( $P = 0.002$ ) gain efficiency by 17%. Gain efficiencies calculated on the basis of carcass-adjusted BW were increased ( $P = 0.003$ ) 26% by feeding ractopamine. Anderson et al. (1989), Carroll et al. (1990), and Preston et al. (1990) showed similar improvements in gain efficiencies due to ractopamine. Schroeder et al. (2003a) and Laudert et al. (2004) demonstrated a 16% improvement in feed efficiency when feedlot steers were fed 20

ppm ractopamine for 28 or 42 d. In a similar study, Schroeder et al. (2003b) reported a 14% improvement in feed efficiencies for feedlot heifers.

Carcass-adjusted daily gain, gain efficiency, and carcass-adjusted gain efficiency were not affected ( $P \geq 0.52$ ) by protein source. For ADG, heifers demonstrated a ractopamine  $\times$  protein source interaction ( $P = 0.04$ ). Heifers not fed ractopamine had greater ADG when fed ESBM than when fed urea, whereas heifers fed ractopamine showed no response ( $P \geq 0.10$ ) to protein supplementation. The response of heifers not receiving ractopamine suggests that performance was improved by increasing MP supply. However, heifers receiving ractopamine showed numerical decreases in daily gains as MP was increased. For heifers fed ractopamine, carcass-adjusted gains were numerically greater when they were fed the urea-supplemented diets, suggesting that there was no benefit to increasing the MP supply. Feedlot steers implanted with trenbolone acetate and estradiol-17 $\beta$  had gain efficiencies that were numerically greater when the diet was supplemented with a combination of 75% feather meal: 25% soybean meal rather than 50:50 or 25:75 of feather meal and soybean meal (Cecava and Hancock, 1994). This suggested that aggressively implanted finishing steers might respond to increases in MP supply, but this response was not observed in our implanted heifers fed ractopamine. Anderson et al. (1989) observed no interaction between dietary ractopamine-HCl (0 to 80 ppm) and dietary protein concentration (11 or 14% CP) for performance of finishing steers over a 56-d period, suggesting that, similar to our results, ractopamine feeding did not markedly increase the MP requirement.

Protein source did not alter HCW ( $P = 0.65$ ), but HCW was increased ( $P = 0.008$ ) 6.9 kg when heifers were fed ractopamine. Schroeder et al. (2003b) observed a significant increase in HCW from heifers fed ractopamine, with an improvement of 2.9 kg. Schroeder et al. (2003a), Laudert et al. (2004), and Carroll et al. (1990) showed similar increases in HCW of feedlot steers fed 20 ppm ractopamine (6.4, 5.6, and 4.9 kg).

Final BW were 8.3 kg greater when heifers were fed ractopamine ( $P < 0.02$ ). Heifers also demonstrated a ractopamine  $\times$  protein source interaction ( $P = 0.04$ ) for final BW that was similar to that observed for ADG. Heifers not fed ractopamine had greater final BW when fed ESBM than when fed urea, whereas heifers fed ractopamine showed no response ( $P \geq 0.10$ ) to protein supplementation. Data reported from previous studies showed a 7.2-kg (Schroeder et al., 2003a) and 6.7-kg (Laudert et al., 2004) increase in final BW of feedlot steers and a 6.6-kg



(Schroeder et al., 2003b) increase in feedlot heifers in response to 20 ppm ractopamine fed for 28 or 42 d.

Carcass data for heifers are shown in Table 2.2. Dressing percentage, LM area, 12th-rib fat thickness, USDA yield grade, and marbling score were not affected by ractopamine or protein source ( $P \geq 0.07$ ). This is in agreement with Schroeder et al. (2003b), who showed no response to ractopamine in feedlot heifers for dressing percentage, 12th-rib fat thickness, LM area, marbling score, USDA yield grade, or USDA quality grade. The KPH of control heifers was least when SSBM was fed, whereas the KPH of heifers fed ractopamine was least when ESBM was fed (ractopamine  $\times$  protein source interaction,  $P = 0.002$ ). These differences are unlikely to be of biological importance. In our experiment, modest responses to the treatments for carcass characteristics would not be detected due to the number of heifers studied. Previous research showed no effect of ractopamine on KPH in feedlot steers (Schroeder et al., 2003a; Laudert et al., 2004) or feedlot heifers (Schroeder et al., 2003b). Schroeder et al. (2003a) and Carroll et al. (1990) reported that ractopamine fed to feedlot steers significantly increased dressing percentage and LM area. These same responses in dressing percentage and LM area were also observed in feedlot heifers (Laudert et al., 2004). With ractopamine feeding, lean muscle deposition increases, potentially leading to increases in LM area (Mersmann, 1998).

Plasma metabolites are presented in Table 2.3. Because differences among treatments existed before the experiment, changes in plasma metabolite concentrations after implementation of treatments were calculated and considered most appropriate for assessing treatment responses. The decrease in plasma glucose concentrations from before the experiment to d 13 was greater when heifers were fed ractopamine ( $P < 0.05$ ). Changes from pretreatment concentrations of plasma lactate were not different ( $P \geq 0.14$ ) among treatments.

Treatment effects on changes from pretreatment concentrations were not observed for plasma urea (Table 2.3). Shifting dietary N from DIP to UIP might be expected to decrease plasma urea concentrations as less ammonia is absorbed across the gut. Urea production, however, is related to N intake above anabolic requirements, and our diets were isonitrogenous and yielded no effect on heifer growth. The effects of ractopamine on plasma urea, if any, may have been difficult to detect because the increase in N deposition in response to ractopamine was only about 5% of N intake (the 0.29 kg/d increase in ADG would correspond to increases in deposition of N of about 9 g/d). Total  $\alpha$ -amino N could be expected to increase with increases in

MP supply, but differences among diets were not observed. Decreases from pretreatment concentrations for plasma total  $\alpha$ -amino N were greater for heifers fed ractopamine ( $P = 0.004$ ), suggesting that more AA were taken up by tissues in response to this growth promotant.

Our diets were formulated to provide different amounts of MP to the heifers, with the urea diet providing the least and the ESBM diet providing the most. Metabolizable protein balances for the urea, SSBM, and ESBM diets were calculated with models from Level 1 of the NRC (1996) and the CNCPS, and the results are shown in Table 2.4. Both models predicted energy allowable gains that were less than those observed. This is likely due to our heifers being individually fed in small pens, thereby minimizing activity of the heifers and reducing their nonproductive energy usage. Because gains for the heifers were under predicted, MP requirements would also be under predicted, and MP balance would be over predicted. Prediction of MP balance by the NRC model showed that the urea diet was slightly deficient, but MP balances were positive with SSBM and more so with ESBM. Predictions from the CNCPS showed all diets to be in a positive MP balance. Lesser MP supply from the urea diet than from the SSBM and ESBM diets was also predicted. The more positive MP balances predicted by the CNCPS than by the NRC can be attributed to 1) the lesser gain predicted by CNCPS, which results in lesser MP requirements, and 2) greater predictions of MP supply by the CNCPS, which resulted from greater predictions of microbial protein supply. Both the NRC and CNCPS predicted the greatest ruminal N balance for the urea diet and the least for the ESBM diet, although the NRC predicted a 121 g/d difference among diets and the CNCPS predicted only a 19 g/d difference. Because ruminal N balance was predicted by the CNCPS to be negative for the ESBM diet, MP balance predicted by the CNCPS did not differ much between the SSBM diet and the ESBM diet. In contrast, the NRC model, which predicted positive ruminal N balances for all diets, predicted a greater difference between the SSBM and ESBM diets. Predictions for MP requirements are based on heifers fed no ractopamine; MP requirements for heifers fed ractopamine would be greater if the efficiency of MP use for tissue gain is not impacted by the ractopamine.

When not fed ractopamine, finishing steers generally do not respond to increases in MP supply above that provided by typical finishing diets containing little UIP supplementation (Gleghorn et al., 2004; Milton et al., 1997). McCoy et al. (1998) demonstrated that steer calves fed a diet based on dry-rolled corn or dry-rolled corn and wet corn gluten feed for 168 d showed

no improvements in performance when supplemented with UIP (80% feather meal and 20% blood meal). Barajas and Zinn (1998) observed minimal improvements in performance of heifers fed diets based on dry-rolled or steam-flaked corn when they were supplemented with cottonseed meal (10% of diet DM) during a 110-d finishing trial. Although research evaluating the effect of MP supply on performance in finishing heifers is limited, MP requirements of heifers are predicted to be less than those of steers due to the lesser amount of protein deposited (NRC, 2000).

It was unknown whether heifers fed ractopamine might respond to increases in MP supply because ractopamine increases growth rate and lean tissue deposition, which in turn might lead to a greater need for MP. Our study demonstrates that heifers fed ractopamine did not respond to increased MP supply. Thus, provision of adequate DIP to the diet by the addition of urea seems sufficient to meet the MP needs of ractopamine-fed heifers consuming typical finishing diets based on steam-flaked corn.

Our results show that ractopamine fed for 28 d before slaughter to heifers implanted with trenbolone acetate/estradiol can improve daily gains and feed efficiency with little impact on carcass characteristics. Our data do not support the concept that dietary metabolizable protein supply needs to be increased above that present in feedlot diets based on steam-flaked corn with urea as the primary nitrogen supplement to maximize the response of finishing heifers to ractopamine.

## References

- Anderson, D. B., E. L. Veenhuizen, J. F. Wagner, M. I. Wray, and D. H. Mowrey. 1989. The effect of ractopamine hydrochloride on nitrogen retention, growth performance, and carcass composition of beef cattle. *J. Anim. Sci.* 67(Suppl. 1):222. (Abstr.)
- Barajas, R., and R. A. Zinn. 1998. The feeding value of dry-rolled and steam-flaked corn in finishing diets for feedlot cattle: Influence of protein supplementation. *J. Anim. Sci.* 76:1744-1752.
- Carroll, L. H., S. B. Laudert, J. C. Parrott, D. H. Mowrey, D. R. White, D. B. Anderson, and J. K. Merrill. 1990. Ractopamine HCl dose titration in feedlot steers: Performance and carcass traits. *J. Anim. Sci.* 68(Suppl. 1):294. (Abstr.)
- Cecava, M. J., and D. L. Hancock. 1994. Effects of anabolic steroids on nitrogen metabolism and growth of steers fed corn silage and corn-based diets supplemented with urea or combinations of soybean meal and feather meal. *J. Anim. Sci.* 72:515-522.
- Fox, D. G., C. J. Sniffen, J. D. O'Connor, J. B. Russell, and P. J. Van Soest. 1992. A net carbohydrate and protein system for evaluating cattle diets: III. Cattle requirements and diet adequacy. *J. Anim. Sci.* 70:3578-3596.
- Galyean, M. L. 1996. Protein levels in beef cattle finishing diets: Industry application, university research, and system results. *J. Anim. Sci.* 74:2860-2870.
- Gleghorn, J. F., N. A. Elam, M. L. Galyean, G. C. Duff, N. A. Cole, and J. D. Rivera. 2004. Effects of crude protein concentration and degradability on performance, carcass characteristics, and serum urea nitrogen concentrations in finishing beef steers. *J. Anim. Sci.* 82:2705-2717.
- Gleghorn, J. F., N. A. Elam, M. L. Galyean, G. C. Duff, N. A. Cole, J. D. Rivera. 2004. Effects of crude protein concentration and degradability on performance, carcass characteristics, and serum urea nitrogen concentrations in finishing beef steers. *J. Anim. Sci.* 82:2705-2717.
- Laudert, S. B., G. J. Vogel, A. L. Schroeder, W. J. Platter, and M. T. Van Koeving. 2004. The effect of Optaflexx on growth performance and carcass traits of steers. Optaflexx Exchange No. 4. Elanco Animal Health, Greenfield, IN.

- Marsh, W. H., B. Fingerhut, and H. Miller. 1965. Automated and manual direct methods for the determination of blood urea. *Clin. Chem.* 11:624-627.
- McCoy, R. A., R. A. Stock, T. J. Klopfenstein, D. H. Shain, and M. J. Klemesrud. 1998. Effect of energy source and escape protein on receiving and finishing performance and health of calves. *J. Anim. Sci.* 76:1488-1498.
- Mersmann, H. J. 1998. Overview of the effects of  $\beta$ -adrenergic receptor agonists on animal growth including mechanisms of action. *J. Anim. Sci.* 76:160-172.
- Milton, C. T., R. T. Brandt, Jr., E. C. Titgemeyer, and G. L. Kuhl. 1997. Effect of degradable and escape protein and roughage type on performance and carcass characteristics of finishing yearling steers. *J. Anim. Sci.* 75:2834-2840.
- Mitchell, A. D., M. B. Solomon, and N. C. Steele. 1991. Influence of level of dietary protein or energy on effects of ractopamine in finishing swine. *J. Anim. Sci.* 69:4487-4495.
- NRC. 1996. *Nutrient Requirements of Beef Cattle: 7th Rev. Ed.: Update 2000.* Natl. Acad. Press, Washington, DC.
- Palmer, D. W., and T. Peters, Jr. 1969. Automated determination of free amino groups in serum and plasma using 2, 4, 6-trinitrobenzene sulfonate. *Clin. Chem.* 15:891-901.
- Preston, R. L., S. J. Bartle, and L. H. Carroll. 1990. Feedlot performance of steers fed ractopamine-hydrochloride. *J. Anim. Sci.* 68(Suppl. 1):276. (Abstr.)
- Russell, J. B., J. D. O'Connor, D. G. Fox, P. J. Van Soest, and C. J. Sniffen. 1992. A net carbohydrate and protein system for evaluating cattle diets: I. Ruminal fermentation. *J. Anim. Sci.* 70:3551-3561.
- Schroeder, A. L., D. M. Polser, S. B. Laudert, and G. J. Vogel. 2003a. The effect of Optaflexx on growth performance and carcass traits of steers. Optaflexx Exchange No. 1. Elanco Animal Health, Greenfield, IN.
- Schroeder, A. L., D. M. Polser, S. B. Laudert, and G. J. Vogel. 2003b. The effect of Optaflexx on growth performance and carcass traits of heifers. Optaflexx Exchange No. 2. Elanco Animal Health, Greenfield, IN.
- Sniffen, C. J., J. D. O'Connor, P. J. Van Soest, D. G. Fox, and J. B. Russell. 1992. A net carbohydrate and protein system for evaluating cattle diets: II. Carbohydrate and protein availability. *J. Anim. Sci.* 70:3562-3577.

Xiao, R. J., Z. R. Xu, and H. L. Chen. 1999. Effects of ractopamine at different dietary protein levels on growth performance and carcass characteristics in finishing pigs. *Anim. Feed Sci. Technol.* 79:119-127.

**Table 2.1** Composition of the diets fed to finishing heifers

Item	Urea	Solvent soybean meal	Expeller soybean meal
Ingredient		----- % of DM -----	
Steam-flaked corn	82.4	77.0	75.7
Concentrated separator byproduct	6.0	6.0	6.0
Alfalfa hay	6.0	6.0	6.0
Urea	1.5	0.5	0.5
Solvent soybean meal	–	6.6	–
Expeller soybean meal	–	–	7.9
Limestone	1.5	1.4	1.4
Salt	0.3	0.3	0.3
Vitamin/mineral premix <sup>1</sup>	0.1	0.1	0.1
Drug premix <sup>2</sup>	2.2	2.2	2.2
Nutrient, calculated			
CP	13.7	13.7	13.7
Degradable intake protein, <sup>3</sup> % of CP	69.3	62.7	57.3
NEm, <sup>3</sup> Mcal/kg	1.45	1.46	1.46
NEg, <sup>3</sup> Mcal/kg	2.36	2.37	2.37
Ca	0.70	0.70	0.70
P	0.26	0.29	0.29
K	1.05	1.18	1.18

<sup>1</sup> Provided (per kg diet DM) 60 mg Mn, 60 mg Zn, 10 mg Cu, 0.6 mg I, 0.25 mg Se, 0.1 mg

Co, and 2,650 IU vitamin A.

<sup>2</sup> Fed to provide (mg/heifer daily): ractopamine-HCl (0 or 200 depending on treatment), melengestrol acetate (0.5), monensin (300), and tylosin (90).

<sup>3</sup> Based on NRC (1996).

**Table 2.2** Effect of ractopamine and dietary protein source on growth and carcass characteristics of finishing heifers

Item	No ractopamine			Ractopamine, 200 mg/d			SEM
	Urea	Soybean Meal	Expeller soybean meal	Urea	Solvent soybean meal	Expeller Soybean meal	
Performance							
Initial BW, kg	475.9	474.9	475.4	475.6	475.4	475.6	6.5
Final BW, <sup>1,2</sup> kg	515.1 <sup>a</sup>	519.8 <sup>ab</sup>	527.8 <sup>bc</sup>	535.2 <sup>c</sup>	527.6 <sup>bc</sup>	524.9 <sup>abc</sup>	4.5
DMI, kg/d	8.55	8.53	9.01	9.14	8.89	8.29	0.35
ADG, <sup>1,2</sup> kg	1.37 <sup>a</sup>	1.53 <sup>ab</sup>	1.81 <sup>bc</sup>	2.06 <sup>c</sup>	1.80 <sup>bc</sup>	1.71 <sup>abc</sup>	0.16
G:F <sup>1</sup>	0.157	0.178	0.198	0.222	0.201	0.206	0.013
Carcass adjusted performance <sup>3</sup>							
ADG, <sup>1</sup> kg	1.59	1.49	1.66	2.14	1.91	1.87	0.18
G:F <sup>a1</sup>	0.185	0.175	0.184	0.234	0.225	0.225	0.020
Carcass performance							
Hot carcass wt, <sup>1</sup> kg	318.0	316.4	319.3	327.8	323.7	322.9	3.2
Dressing percentage <sup>4</sup>	61.7	60.9	60.5	61.4	61.5	61.5	0.53
KPH, <sup>2</sup> %	2.15 <sup>c</sup>	1.96 <sup>ab</sup>	2.17 <sup>c</sup>	2.13 <sup>bc</sup>	2.17 <sup>c</sup>	1.92 <sup>a</sup>	0.06
LM area, cm <sup>2</sup>	91.5	83.5	81.6	86.1	90.4	91.3	3.5
12th-rib fat, cm	0.69	0.66	0.76	0.74	0.85	0.76	0.09
USDA Yield grade	1.73	2.05	2.31	2.13	2.00	1.81	0.20
Marbling score <sup>5</sup>	338	390	346	344	338	348	22

<sup>a-c</sup> Within a row, means without a common superscript letter differ,  $P < 0.05$ .

<sup>1</sup> Effect of ractopamine,  $P < 0.05$ .

<sup>2</sup> Ractopamine  $\times$  protein interaction,  $P < 0.05$ .

<sup>3</sup> Computed by using final BW = hot carcass wt/0.61.

<sup>4</sup> Dressing percentage = (HCW/BW)  $\times$  100.

<sup>5</sup> Slight = 300 – 399.



**Table 2.3** Effect of ractopamine and dietary protein source on plasma metabolites in finishing heifers

Plasma, mM	No ractopamine			Ractopamine, 200 mg/d			SEM
	Urea	Solvent soybean meal	Expeller soybean meal	Urea	Solvent soybean meal	Expeller soybean meal	
<b>Urea</b>							
Pre-treatment	4.57	5.41	5.49	4.80	4.81	5.13	0.34
Post-treatment <sup>1,2,3</sup>	4.07	5.19	5.06	4.22	4.30	4.50	0.27
Difference	-0.50	-0.22	-0.43	-0.58	-0.51	-0.63	0.28
<b>Total α-amino nitrogen</b>							
Pre-treatment	2.98	2.97	3.11	3.21	3.02	3.10	0.11
Post-treatment <sup>1,2,4</sup>	2.85 <sup>abc</sup>	3.05 <sup>bc</sup>	3.14 <sup>c</sup>	2.95 <sup>abc</sup>	2.82 <sup>ab</sup>	2.71 <sup>a</sup>	0.11
Difference <sup>2</sup>	-0.13	0.08	0.03	-0.27	-0.20	-0.39	0.12
<b>Glucose</b>							
Pre-treatment <sup>2,4</sup>	6.81 <sup>b</sup>	5.20 <sup>a</sup>	5.08 <sup>a</sup>	5.89 <sup>ab</sup>	6.71 <sup>b</sup>	7.01 <sup>b</sup>	0.49
Post-treatment <sup>1</sup>	6.56	5.48	5.02	5.24	5.90	5.95	0.37
Difference <sup>2</sup>	-0.25	0.28	-0.06	-0.65	-0.81	-1.06	0.44
<b>Lactate</b>							
Pre-treatment	5.63	4.38	3.83	5.07	6.13	6.74	0.94
Post-treatment <sup>1</sup>	4.69	5.04	3.44	4.02	4.58	5.58	0.79
Difference	-0.94	0.66	-0.39	-1.05	-1.56	-1.16	0.88

<sup>a-c</sup> Within a row, means without a common superscript letter differ,  $P < 0.05$ .

<sup>1</sup> Blood samples collected 13 d after initiation of ractopamine feeding.

<sup>2</sup> Effect of ractopamine,  $P < 0.05$ .

<sup>3</sup> Effect of protein, expeller soybean meal = solvent soybean meal > urea,  $P < 0.05$

<sup>4</sup> Ractopamine × protein interaction,  $P < 0.05$

**Table 2.4** Metabolizable protein balances of heifers fed diets with different protein supplements

Item	Urea	Solvent soybean meal	Expeller soybean meal
Prediction from NRC (1996), Level 1			
Energy allowable gain, kg/d	1.36	1.37	1.37
MP required, g/d	697	698	698
MP from diet, g/d	688	761	808
MP from bacteria, g/d	404	406	404
MP from UIP, g/d <sup>1</sup>	284	355	404
MP balance, g/d	-8	63	109
Ruminal N balance, g/d	169	109	48
Prediction from Cornell Net Carbohydrate and Protein System 5.0 <sup>2</sup>			
Energy allowable gain, kg/d	1.22	1.23	1.20
MP required, g/d	623	629	631
MP from diet, g/d	877	913	918
MP from bacteria, g/d	625	618	564
MP from UIP, g/d <sup>1</sup>	253	295	354
MP balance, g/d	254	284	287
Ruminal N balance, g/d	4	0	-15
Ruminal peptide balance, g/d	-53	-22	-33

<sup>1</sup> UIP = undegradable intake protein.

<sup>2</sup> Cornell University, Ithaca, NY.

**CHAPTER 3 - Effects of Steroidal Implantation and Ractopamine-HCl on Nitrogen Retention, Blood Metabolites, and Longissimus mRNA Expression of IGF-I in Holstein Steers**

D. K. Walker, E. C. Titgemeyer, E. K. Sissom, K. R. Brown, J. J. Higgins,  
and B. J. Johnson

Used by permission of the Journal of Animal Physiology and Animal Nutrition, 2007  
91:439-447.

## Summary

Six Holstein steers ( $231 \pm 17$  kg) housed in metabolism crates were used in a randomized complete block design with three blocks of two steers based on previous serum insulin-like growth factor (IGF)-I concentrations. One of the two steers in each block was implanted with 120 mg trenbolone acetate and 24 mg estradiol-17 $\beta$  on day 0. None of the steers were fed ractopamine-HCl the initial 28 days, and then all steers were fed 200 mg of ractopamine-HCl per steer daily from day 28 until the end of the trial. Steers were fed a corn-based diet (62% rolled corn, 20% expeller soya bean meal and 15% alfalfa hay) twice daily with an average dry matter intake of 4.8 kg/day. Blood and M. longissimus biopsy samples were collected prior to implantation and on days 14, 28, 42 and 56. There was an implant  $\times$  ractopamine interaction for retained nitrogen ( $p < 0.05$ ); ractopamine feeding led to only small improvements in nitrogen retention for implanted steers (45.9 vs. 44.5 g/day), whereas ractopamine led to larger increases in nitrogen retention for non-implanted steers (39.0 vs. 30.4 g/day). Implantation increased ( $p < 0.05$ ) and ractopamine tended to decrease ( $p = 0.06$ ) serum IGF-I concentrations. Implantation tended to increase ( $p = 0.16$ ) and ractopamine decreased ( $p < 0.05$ ) mRNA expression of IGF-I in the M. longissimus. Ractopamine decreased mRNA expression of  $\beta$ 1- and  $\beta$ 2-receptors in M. longissimus ( $p \leq 0.02$ ). The steroidal implant and the feeding of ractopamine both increased nitrogen retention in steers, but the combination did not yield an additive response. The two growth promotants had opposite effects on serum concentrations of IGF-I and mRNA expression of IGF-I in M. longissimus.

## Introduction

Steroidal implants that contain trenbolone acetate (TBA)/estradiol-17 $\beta$  (E<sub>2</sub>) improved average daily gain and gain efficiency (Johnson et al., 1996a), increased serum insulin-like growth factor (IGF)-I (Johnson et al., 1996b; Dunn et al., 2003; Pampusch et al., 2003) and increased IGF-I mRNA expression in the liver of wethers and *M. longissimus* of steers (Johnson et al., 1998a; Dunn et al., 2003; White et al., 2003). Additionally, satellite cells prepared from *M. semimembranosus* of steers implanted with TBA/E<sub>2</sub> have a shorter lag phase when cultured compared with satellite cells collected from non-implanted steers, indicating a greater number of proliferating satellite cells in muscle of implanted steers (Johnson et al., 1998b). Satellite cells that withdraw from the cell cycle fuse with existing myofibres, supporting myofibre hypertrophy. These data suggest that TBA/E<sub>2</sub> improves muscle growth by its effects on circulating and local tissue concentrations of IGF-I and, subsequently, on satellite cell proliferation.

Ractopamine is predominantly a  $\beta_1$ -adrenergic agonist that has binding affinity for both  $\beta_1$ - and  $\beta_2$ -adrenergic receptors (Colbert et al., 1991). Binding of ractopamine to the  $\beta$ -adrenergic receptors elicits a response that results in increased lean muscle mass with little or no effect on adipose tissue deposition (Liu et al., 1994; Mersmann, 1998). Ractopamine can improve daily gains and gain efficiencies by 17% and 18% in steers (Laudert et al., 2004) and by 18% and 16 to 18% in heifers (Schroeder et al., 2003; Walker et al., 2006). Unlike steroidal implants, cimaterol (a  $\beta_2$ -agonist) did not affect DNA content in the *M. semitendinosus* in rams, although it increased the total RNA concentration of *M. semitendinosus* by 109% (O'Connor et al. 1991). Therefore, it appears that cimaterol had no impact on satellite cell proliferation, and the mechanism by which cimaterol induces skeletal muscle hypertrophy may be different from that of steroidal implants.

With potential differences in mode of action between steroidal implants and  $\beta$ -agonists, additive or synergistic responses might be expected. Our objective was to determine the effect of a TBA/E<sub>2</sub> implant and of feeding ractopamine on several growth-related criteria in steers.

## Materials and Methods

Procedures for this study were approved by the Kansas State University Institutional Animal Care and Use Committee.

#### *Animals and experimental design*

Six Holstein steers (initially weighing  $231 \pm 17$  kg and 6 months of age) castrated at 30 days of age were used to evaluate the interaction between steroidal implantation and feeding ractopamine. Steers were housed in individual metabolism crates in a temperature-controlled room (21°C) with continuous lighting. Prior to the study, steers were adapted to the diet (Table 3.1) for 1 week. All steers had free access to water and were limit-fed the same diet (4.3 to 5.4 kg/day of dry matter) in equal proportions at 12-h intervals; steers in each block were fed the same amount of feed.

The experiment was arranged as a randomized complete block design with three blocks of two steers based on previous serum IGF-I concentrations with one steer in each block implanted with 120 mg TBA and 24 mg E<sub>2</sub> (Revalor S<sup>®</sup>; Intervet, Millsboro, DE, USA) on day 0 and the others not implanted. None of the steers were fed ractopamine-HCl the initial 28 days, and then all steers were fed 200 mg per steer daily of ractopamine-HCl (Optaflexx<sup>®</sup>; Elanco Animal Health, Greenfield, IN, USA) beginning on the evening of day 28 and continuing through the end of the trial on days 56, 57, or 58.

#### *Nitrogen retention and diet digestibility*

Representative samples of the diet were collected daily, composited over 4-day periods and stored (−20 °C). Orts, if any, were collected, composited by steer over 4-day periods and stored (−20 °C). Faeces and urine for each steer were collected daily and weighed to determine the total output. Urine was collected in buckets containing 300 ml of 6 M HCl to prevent ammonia loss. Representative samples of faeces (5%) and urine (1%) were saved, composited by steer over 4-day periods, and stored (−20 °C). Collections were initiated 4 days before implantation to provide pretreatment values. The first collection of urine and feces after ractopamine feeding was initiated on day 29. Samples of the diet, Orts and faeces were analyzed for dry matter (105 °C in forced-air oven for 24 h) and nitrogen using a Leco FP 2000 nitrogen analyzer (Leco Corp., St. Joseph, MI, USA). Urine was analyzed for ammonia and urea concentrations colorimetrically (Technicon Industrial Systems, Buffalo Grove, IL, USA; Method No. 337-74T and 339-01, respectively).

#### *Blood metabolites and hormones*

Jugular blood samples were collected 2 h after the morning feeding on days 0 (before implantation), 14, 28 (before ractopamine was fed), 42 and 56. Blood was collected into vacuum tubes (Becton Dickinson, Franklin Lakes, NJ, USA) containing sodium heparin, immediately placed on ice and centrifuged for 20 min at  $1\,000 \times g$  to obtain plasma. Blood was also collected into vacuum tubes without additives, allowed to clot for 24 h at 4 °C and then centrifuged for 20 min at  $1\,000 \times g$  to obtain serum. Plasma samples were stored ( $-20\text{ °C}$ ) for later analysis of glucose (Gochman and Schmitz, 1972) and urea (Marsh et al., 1965). Sera were stored ( $-20\text{ °C}$ ) for later analysis of insulin (radioimmunoassay kit, DSL-1600; Diagnostic Systems Laboratories, Webster, TX, USA; intra-assay CV = 5.3%, assay sensitivity = 0.02 ng/ml; Greenwood et al., 2001) and IGF-I (IGF-I coated-tube immunoradiometric assay kit, DSL-5600; Diagnostic Systems Laboratories; intra-assay CV = 3.4%, assay sensitivity = 5.0 ng/ml; Greenwood et al., 2001).

#### *Biopsy samples from M. longissimus*

Biopsy samples were collected from the *M. longissimus* from each steer as described by Dunn et al. (2003) and Pampusch et al. (2003) on days 0 (before implantation), 14, 28 (before ractopamine was fed), 42 and 56. Biopsies on days 0, 28 and 56 were collected from the right side and on days 14 and 42 from the left side, with sampling sites being initially at the last rib and moving 5 cm anterior to the previous site.

#### *RNA isolation*

Muscle biopsy samples (0.5 g) from each steer were homogenized in 10 ml of a 5 M guanidine thiocyanate, 50 mM Tris-HCl, 25 mM EDTA, 0.5% lauryl sarcosine and 1%  $\beta$ -mercaptoethanol solution (Solution D), followed by rapid freezing in liquid nitrogen and storage at  $-80\text{ °C}$  for later RNA isolation. Total RNA was isolated as described by Dunn et al. (2003) and Pampusch et al. (2003). Samples were treated with DNase to remove any contaminating genomic DNA using a commercially available kit (DNA-free<sup>®</sup>; Ambion, Austin, TX, USA). The concentration of RNA was determined by absorbance at 260 nm. Electrophoresis of total RNA through a 1% agarose-formaldehyde gel followed by ethidium bromide staining to allow visualization of 28S and 18S ribosomal RNA (rRNA) was used to assess the integrity of RNA. One microgram of total RNA was then reverse-transcribed to produce the first-strand complementary DNA (cDNA) using TaqMan<sup>®</sup> reverse transcriptase (Applied Biosystems, Foster

City, CA, USA) following the protocol recommended by the manufacturer. Random hexamers were used as primers in cDNA synthesis.

#### *Day 56 muscle and liver samples*

Steers were killed by administration of sodium pentobarbital anesthesia followed by exsanguination in groups of two steers from the same block on days 56, 57 and 58. All steers received ractopamine until harvested. Following exsanguination, M. semimembranosus and liver samples were collected; these samples are referred to as day 56 samples throughout this paper. Using sterile techniques, 50 g of M. semimembranosus and liver were dissected, rapidly frozen in liquid nitrogen and stored at  $-80^{\circ}\text{C}$  for subsequent RNA isolation. The RNA was isolated from liver samples by using the RNeasy<sup>®</sup> Mini Kit (Qiagen; Valencia, CA, USA). The RNA was isolated from M. semimembranosus samples by using TRI REAGENT<sup>™</sup> (Sigma; St. Louis, MO, USA). Methods for determining RNA concentration and RNA integrity and for synthesizing cDNA were as described above.

#### *Real-time polymerase chain reaction*

Real-time quantitative-polymerase chain reaction was used to measure the quantity of mRNA for  $\beta_1$ -,  $\beta_2$ - and  $\beta_3$ -adrenergic receptors, IGF-I (Class 1) and 18S rRNA in total RNA isolated from M. longissimus and M. semimembranosus. The quantity of mRNA for IGF-I (Class 1) and 18S rRNA was measured in total RNA isolated from liver. Measurement of the relative quantity of cDNA was conducted using TaqMan<sup>®</sup> Universal PCR Master Mix (Applied Biosystems), 900 nM of the appropriate forward and reverse primers, 200 nM of the appropriate TaqMan<sup>®</sup> detection probe and 1  $\mu\text{l}$  of the cDNA mixture. Sequences for primers and probes for IGF-I and  $\beta_1$ -,  $\beta_2$ - and  $\beta_3$ - receptors are presented in Table 3.2. The primers for IGF-I were designed to estimate Class 1 IGF-I mRNA. Specifically, primers for IGF-I spanned exon 1 and exon 3 which estimated only Class 1 IGF-I mRNA. Commercially available eukaryotic 18S rRNA primers and probes were used as an endogenous control (Applied Biosystems; Genbank Accession no. X03205). Assays were performed in an ABI Prism<sup>®</sup> 7000 sequence detection system (Applied Biosystems) using thermal cycling parameters recommended by the manufacturer (50 cycles of 15 sec at  $95^{\circ}\text{C}$  and 1 min at  $60^{\circ}\text{C}$ ). Relative expressions of mRNA for  $\beta_1$ -,  $\beta_2$ - and  $\beta_3$ -receptors and IGF-I were normalized to the 18S rRNA endogenous control and expressed in arbitrary units.

#### *Statistical analyses*



Data were analyzed using the MIXED procedure of SAS System for Windows Release 8.1 (SAS Inst. Inc., Cary, NC, USA). The model contained the effects of implant, ractopamine, day within ractopamine, implant × ractopamine, and implant × day within ractopamine. Block and block × implant were included as random variables. For nitrogen retention data, pretreatment values were included as a covariate. Treatment means were computed using the LSMEANS option. The LSD procedure was used to separate means.

## Results

### *Nitrogen retention and diet digestibility*

Data for nitrogen retention and diet digestibility are presented in Table 3.3. Dry matter digestibility was increased by ractopamine. Ractopamine decreased nitrogen intake, fecal nitrogen output and urinary urea nitrogen output relative to controls. Urinary nitrogen output and urinary ammonia nitrogen output were decreased by implantation and by feeding ractopamine to control steers, but were not affected when ractopamine was fed to implanted steers (significant implant × ractopamine interactions). Similarly, nitrogen retention was increased by implantation during the initial 28 days of the study and by feeding ractopamine to control steers, but was not affected by feeding ractopamine to implanted steers (significant implant × ractopamine interaction).

### *Blood metabolites*

Data for blood metabolites are presented in Fig. 3.2. Plasma urea concentrations were similar among treatments. Ractopamine tended to decrease plasma glucose concentrations, although the change was only 4%. Serum insulin concentrations were not affected by implantation, but tended ( $p = 0.13$ ) to be decreased by ractopamine with the decrease being more dramatic in the implanted steers. Serum IGF-I concentrations were increased by implantation, whereas ractopamine tended to decrease serum IGF-I concentrations.

### *Messenger RNA expression in *M. longissimus*, *M. semimembranosus* and liver*

Implantation tended ( $p = 0.16$ ) to increase IGF-I mRNA expression in *M. longissimus* (Fig. 3.3), whereas ractopamine decreased IGF-I mRNA expression in *M. longissimus* in all steers. Messenger RNA expression of IGF-I in *M. semimembranosus* also tended to be greater in implanted steers than in control steers (Table 3.4). Implantation tended to increase hepatic IGF-I mRNA expression compared to control steers (Table 3.4). Data for mRNA expression of

$\beta_1$ -,  $\beta_2$ - and  $\beta_3$ - receptors in *M. longissimus* are presented in Fig. 3.3. Implantation had no effect on mRNA expression of  $\beta_1$ -,  $\beta_2$ - and  $\beta_3$ -receptors in *M. longissimus*, but expression of  $\beta_1$ - and  $\beta_2$ -receptor mRNA was decreased by ractopamine. Implantation did not affect  $\beta_1$ - or  $\beta_3$ -receptor mRNA expression in the *M. semimembranosus* (Table 3.4), but  $\beta_2$ -receptor mRNA expression tended to be increased by implantation.

## Discussion

A change in digestibility in response to ractopamine feeding was observed in our study. Brikas (1989) suggested that  $\beta$ -agonists act on  $\beta$ -receptors in the gastrointestinal tract and decrease gut contractions. Subsequently, retention time of digesta is increased resulting in increased digestion. Ractopamine decreased nitrogen intake, but the decrease in nitrogen intake was a result of a lesser concentration of nitrogen in the individual feed ingredients when ractopamine was fed and not a change in dry matter intake.

As shown in Fig. 3.1, implantation increased nitrogen retention within the first 4 days after implantation. When ractopamine feeding was initiated, it led to rapid increases in nitrogen retention within the first 4 days, but the increase was more dramatic in the control steers than in the implanted steers such that the difference in nitrogen retention between the control and implanted steers was less when ractopamine was fed (Fig. 3.1). Loblely et al. (1985) demonstrated that nitrogen retention in steers implanted with 140 mg TBA/ 20 mg E<sub>2</sub> was more than double that of nonimplanted steers with large responses observed even 11 weeks after implantation. Similar to our responses to ractopamine, Anderson et al. (1989) reported a 51% increase in nitrogen retention in steers (287 kg) fed diets containing 100 ppm ractopamine. Our data support previous findings that implantation and ractopamine increase nitrogen retention in steers.

Steers implanted with 140 mg TBA/ 28 mg E<sub>2</sub> demonstrated a 30% decrease in plasma urea concentrations compared to nonimplanted steers (Hongerholt et al., 1992). In contrast, urea concentrations were not affected by treatment in our study. Although insulin concentrations were not significantly affected by ractopamine, implanted steers demonstrated a 66% decrease in concentrations when ractopamine was fed. Experiments conducted by O'Connor et al. (1991) demonstrated decreases in serum insulin concentrations in lambs fed 10 ppm cimaterol for 6

weeks. Although large changes in insulin concentrations were observed, glucose concentrations were not altered and this could likely be a result of changes in glucose sensitivity.

Johnson et al. (1996b) reported in finishing steers that implantation with a TBA/E<sub>2</sub> implant increased circulating IGF-I through 115 days compared to non-implanted controls. In our study, steers were implanted for a total of 56 days and were implanted for 28 days before ractopamine feeding. Similar to our results, Beermann et al. (1987) reported 46.5 and 21.5% decreases in serum IGF-I concentrations in lambs fed cimaterol for 6 and 12 weeks compared to lambs not fed cimaterol. The majority of IGF-I in circulation is thought to be derived from liver production and, therefore, decreases in IGF-I concentrations in the steers fed ractopamine in our study could be attributed to ractopamine affecting hepatic IGF-I synthesis or release.

The possibility exists that responses to ractopamine feeding resulted from confounding with time. The temporal patterns of serum IGF-I (Fig. 3.2) and of *M. longissimus* IGF-I mRNA expression (Fig. 3.3), however, suggest a rather specific response after the initiation of ractopamine feeding without subsequent decreases between days 42 and 56, which would be expected if decreases were related to passage of the time. Johnson et al. (1996b) observed that serum IGF-I decreased over time in both implanted and control steers, but their cattle were yearling steers nearing their physiological end-point, whereas our steers were well less than half of their mature weight. Thus, an age-related depression in IGF-I would be unlikely to explain the decreases in serum IGF-I and mRNA expression of IGF-I by *M. longissimus* in our experiment.

Finishing steers implanted with TBA/E<sub>2</sub> for 40 days had 68% more IGF-I mRNA in *M. longissimus* than did control steers (Johnson et al., 1998a). Pampusch et al. (2003) reported that steers implanted with TBA/E<sub>2</sub> had significantly greater IGF-I mRNA in *M. longissimus* on days 14 and 28 compared to steers not implanted. These data are in agreement with our findings that TBA/E<sub>2</sub> will elevate IGF-I mRNA expression in *M. longissimus* of steers. In contrast to implants, steers fed ractopamine in our study demonstrated an opposite effect in that IGF-I mRNA expression was decreased. Sissom et al. (2005) reported that heifers implanted with 200 mg TBA/20 mg E<sub>2</sub> on day 0 and then fed ractopamine starting on day 154 had significantly less IGF-I mRNA abundance in the *M. semimembranosus* when ractopamine was fed. However, in heifers initially implanted with 80 mg TBA/8 mg E<sub>2</sub> and re-implanted with 200 mg TBA 96 days prior to ractopamine feeding, IGF-I mRNA expression in *M. semimembranosus* was not affected

by ractopamine being fed for 28 days (Sissom et al., 2005). Our samples of *M. semimembranosus* collected on day 56 demonstrated a pattern for IGF-I mRNA expression similar to that in *M. longissimus*. Because all steers received ractopamine during the final 28 days of the experiment, only the effect of implant could be evaluated in *M. semimembranosus*.

Hepatic IGF-I mRNA abundance was greater in implanted steers, which matches the response to implantation for circulating concentrations of IGF-I. These data support that hepatic production of IGF-I was a major contributor to circulating concentrations. Although mRNA expression is not always directly correlated with that translated, mRNA expression of IGF-I presented a pattern consistent with serum IGF-I and with previously observed effects of TBA/E<sub>2</sub> implants on IGF-I mRNA (Pampusch et al., 2003). Increases in local tissue production and/or circulating concentrations of IGF-I can increase muscle growth in implanted animals. From our data, however, it appears that increases in IGF-I are not responsible for enhanced growth with ractopamine; decreases in local tissue production as well as circulating concentrations of IGF-I were observed in response to ractopamine. Therefore, the increases in growth observed in response to ractopamine would seem to involve other mechanisms. It is possible, however, that IGF-I, although lesser in concentration, is more active in ractopamine-treated cattle due to changes in IGF binding proteins (IGFBP) or in the type I IGF-I receptor. Awede et al. (2002) reported that clenbuterol, a  $\beta_2$ -agonist, fed to rats increased mRNA expression of IGF-I and IGFBP-4 in the *M. soleus*, but decreased circulating concentrations of IGF-I. The association of IGFBP-4 with IGF-I could alter its half-life and biological activity. We did not measure any of the IGFBP and, therefore, this suggestion is speculation. Yimlamai et al. (2005) reported that clenbuterol fed to rats for 2 weeks decreased protein expression of IGF-I in the *M. plantaris*, but did not affect protein expression of IGF-I in the *M. tibialis anterior*. These data support that decreases in IGF-I could be mediated by  $\beta$ -agonist compounds, like ractopamine.

Sissom et al. (2005) reported that, in the *M. semimembranosus* of implanted heifers, ractopamine did not affect  $\beta_1$ -receptor mRNA abundance and tended to increase  $\beta_2$ -receptor mRNA expression. These data conflict with our findings that ractopamine decreased abundance of both  $\beta_1$ - and  $\beta_2$ -receptor mRNA in *M. longissimus*. Differences between our study and that of Sissom et al. (2005) include the implant strategy and age and gender of the cattle. Yearling steers implanted with 80 mg TBA/16 mg E<sub>2</sub> and re-implanted with 120 mg TBA/24 mg E<sub>2</sub> between 122 and 164 days before ractopamine feeding had increased  $\beta_2$ -receptor mRNA

expression in *M. semimembranosus* when fed ractopamine for 28 days compared to steers that did not receive ractopamine (Winterholler et al., 2006).

Chronic exposure to  $\beta$ -agonists is suggested to lead to desensitization of the  $\beta$ -receptors (Hausdorff et al., 1990) which is consistent with the decreases in  $\beta_1$ - and  $\beta_2$ -receptor mRNA that we observed in response to ractopamine, although decreases in response to ractopamine were not greater after 28 days than after 14 days of feeding. In our study  $\beta_1$ - and  $\beta_2$ -receptor mRNA abundance was suppressed by ractopamine indicating a down-regulation of the receptors mRNA levels.

Our results demonstrate that implanting steers with TBA/E<sub>2</sub> has a stimulatory effect on serum IGF-I and IGF-I mRNA expression in the *M. longissimus*. In contrast, ractopamine appears to have a different mode of action, as demonstrated by decreases in serum IGF-I and in IGF-I mRNA expression by the *M. longissimus*. Both implanting steers and feeding ractopamine can improve growth, but, in our model with Holstein steers, a combination of the two growth promotants was less than additive for increasing nitrogen retention.

## **Acknowledgements**

Contribution No. 06-188-J from the Kansas Agricultural Experiment Station, Manhattan.

## References

- Anderson, D. B.; Veenhuizen, E. L.; Wagner, J. F.; Wray, M. I.; Mowrey, D. H., 1989: The effect of ractopamine hydrochloride on nitrogen retention, growth performance and carcass composition of beef cattle. *Journal of Animal Science* 67(Suppl. 1), 222.
- Awede, B. L.; Thissen, J. P.; LeBacq, J., 2002: Role of IGF-I and IGF-BPs in the changes of mass and phenotype induced in rat soleus muscle by clenbuterol. *American Journal of Physiology: Endocrinology and Metabolism* 282, E31-E37.
- Beermann, D. H.; Butler, W. R.; Hogue, D. E.; Fishell, V. K.; Dalrymple, R. H.; Ricks, C. A.; Scanes, C. G., 1987: Cimaterol-induced muscle hypertrophy and altered endocrine status in lambs. *Journal of Animal Science* 65, 1514-1524.
- Brikas, P., 1989: The adrenergic receptors in the control of reticulo-ruminal myoelectrical activity in sheep. *Journal of Veterinary Medicine* 36, 402-410.
- Colbert, W. E.; Williams, P. D.; Williams, G. D., 1991:  $\beta$ -adrenergic profile of ractopamine-HCl in isolated smooth and cardiac muscle tissues of rat and guinea pig. *Journal of Pharmacy and Pharmacology* 43, 844-847.
- Dunn, J. D.; Johnson, B. J.; Kayser, J. P.; Waylan, A. T.; Sissom, E. K.; Drouillard, J. S., 2003: Effects of flax supplementation and a combined trenbolone acetate and estradiol implant on circulating insulin-like growth factor-I and muscle insulin-like growth factor-I messenger RNA levels in beef cattle. *Journal of Animal Science* 81, 3028-3034.
- Gochman, N.; Schmitz, J. M., 1972: Application of a new peroxide indicator reaction to the specific, automated determination of glucose with glucose oxidase. *Clinical Chemistry* 18, 943-950.
- Greenwood, R. H.; Titgemeyer, E. C.; Stokka, G. L.; Drouillard, J. S.; Loest, C. A., 2001: Effects of L-carnitine on nitrogen retention and blood metabolites of growing steers and performance of finishing steers. *Journal of Animal Science* 79, 254-260.
- Hausdorff, W. P.; Caron, M. G.; Lefkowitz, R. J., 1990: Turning off the signal: desensitization of  $\beta$ -adrenergic receptor function. *Federation of American Societies for Experimental Biology Journal* 4, 2881-2889.
- Hongerholt, D. D.; Crooker, B. A.; Wheaton, J. E.; Carlson, K. M.; Jorgenson, D. M., 1992: Effects of a growth hormone-releasing factor analogue and an estradiol-trenbolone acetate

- implant on somatotropin, insulin-like growth factor I and metabolite profiles in growing Hereford steers. *Journal of Animal Science* 70, 1439-1448.
- Johnson, B. J.; Anderson, P. T.; Meiske, J. C.; Dayton, W. R., 1996a: Effects of a combined trenbolone acetate and estradiol implant on feedlot performance, carcass characteristics and carcass composition of feedlot steers. *Journal of Animal Science* 74, 363-371.
- Johnson, B. J.; Halstead, N.; White, M. E.; Hathaway, M. R.; DiCostanzo, A.; Dayton, W. R., 1998a: Activation state of muscle satellite cells isolated from steers implanted with a combined trenbolone acetate and estradiol implant. *Journal of Animal Science* 76, 2779-2786.
- Johnson, B. J.; Hathaway, M. R.; Anderson, P. T.; Meiske, J. C.; Dayton, W. R., 1996b: Stimulation of circulating insulin-like growth factor I (IGF-I) and insulin-like growth factor binding proteins (IGFBP) due to administration of a combined trenbolone acetate and estradiol implant in feedlot cattle. *Journal of Animal Science* 74, 372-379.
- Johnson, B. J.; White, M. E.; Hathaway, M. R.; Christians, C. J.; Dayton, W. R., 1998b: Effect of a combined trenbolone acetate and estradiol implant on steady-state IGF-I mRNA concentrations in the liver of wethers and the longissimus muscle of steers. *Journal of Animal Science* 76, 491-497.
- Laudert, S. B.; Vogel, G. J.; Schroeder, A. L.; Platter, W. J.; Van Koevering, M. T., 2004: The effect of Optaflexx on growth performance and carcass traits of steers. Optaflexx™ Exchange No. 4. Elanco Animal Health, Greenfield, IN.
- Liu, C. Y.; Grant, A. L.; Kim, K.-H.; Ji, S. Q.; Hancock, D. L.; Anderson, D. B.; Mills, S. E., 1994: Limitations of ractopamine to affect adipose tissue metabolism in swine. *Journal of Animal Science* 72, 62-67.
- Lobley, G. E.; Connell, A.; Mollison, G. S.; Brewer, A.; Harris, C. I.; Buchan, V.; Galbraith, J., 1985: The effects of a combined implant of trenbolone acetate and oestradiol-17 $\beta$  on protein and energy metabolism in growing beef steers. *British Journal of Nutrition* 54, 681-694.
- Marsh, W. H.; Fingerhut, B.; Miller, H., 1965: Automated and manual direct methods for determination of blood urea. *Clinical Chemistry* 11, 624-627.
- Mersmann, H. J., 1998: Overview of the effects of  $\beta$ -adrenergic receptors on animal growth including mechanism of action. *Journal of Animal Science* 76, 160-172.

- O'Connor, R. M.; Butler, W. R.; Hogue, D. E.; Beermann, D. H., 1991: Temporal pattern of skeletal muscle changes in lambs fed cimaterol. *Domestic Animal Endocrinology* 8, 549-554.
- Pampusch, M. S.; Johnson, B. J.; White, M. E.; Hathaway, M. R.; Dunn, J. D.; Waylan, A. T.; Dayton, W. R., 2003: Time course of changes in growth factor mRNA levels in muscle of steroid-implanted and nonimplanted steers. *Journal of Animal Science* 81, 2733-2740.
- Schroeder, A. L.; Polser, D. M.; Laudert, S. B.; Vogel, G. J., 2003: The effect of Optaflexx on growth performance and carcass traits of heifers. Optaflexx™ Exchange No. 2. Elanco Animal Health, Greenfield, IN.
- Sissom, E. K.; Hutcheson, J. P.; Yates, D. A.; Johnson, B. J., 2005: Effect of implant strategy and Optaflexx administration to feedlot heifers on  $\beta$ -adrenergic receptor and insulin-like growth factor-I messenger RNA (mRNA) abundance. In: Proceedings of 2005 Plains Nutrition Council Spring Conference, Publication No. AREC 05-20 Texas A&M Research and Extension Center, Amarillo, pp. 123-124.
- Walker, D. K.; Titgemeyer, E. C.; Drouillard, J. S.; Loe, E. R.; Depenbusch, B. E.; Webb, A. S., 2006: Effects of ractopamine and protein source on growth performance and carcass characteristics of feedlot heifers. *Journal of Animal Science* 84, 2795-2800.
- White, M. E.; Johnson, B. J.; Hathaway, M. R.; Dayton, W. R., 2003: Growth factor messenger RNA levels in muscle and liver of steroid-implanted and nonimplanted steers. *Journal of Animal Science* 81, 965-972.
- Winterholler, S. J.; Parsons, G. L.; Sissom, E. K.; Hutcheson, J. P.; Swingle, R. S.; Johnson, B. J., 2006: Effect of Optaflexx and days on feed on feedlot performance, carcass characteristics and skeletal muscle gene expression in yearling steers. Beef Cattle Research. Agricultural Experiment Station, Kansas State University, Manhattan, pp. 20-23.
- Yimlamai, T.; Dodd, S. L.; Borst, S. E.; Park, S., 2005: Clenbuterol induces muscle-specific attenuation of trophy though effects on the ubiquitin-proteasome pathway. *Journal of Applied Physiology* 99, 71-80.



**Table 3.1** Diet Composition

Ingredient	% of DM
Dry rolled corn	62.0
Expeller soya bean meal	20.0
Alfalfa hay	15.0
Limestone	1.4
Trace mineral salt*	0.5
Dicalcium phosphate	0.22
Vitamin premix†	0.15
Sulfur	0.05
Rumensin® ‡	0.017
Tylan® §	0.0125

\* Composition (minimum guarantee, %): NaCl (95 to 99); Mn (> 0.24); Cu (> 0.032); Zn (> 0.32); I (> 0.007); and Co (> 0.4).

† Provided 4,400 IU of vitamin A; 2,200 IU of vitamin D; and 35 IU of vitamin E per kg of diet DM.

‡ Provided 30 mg of monensin/kg of diet DM.

§ Provided 11 mg of tylosin/kg of diet DM.

**Table 3.2** Sequence of bovine specific polymerase chain reaction primers and TaqMan probes used for determination of expression of mRNA of IGF-I, and  $\beta_1$ -,  $\beta_2$ -, and  $\beta_3$ -adrenergic receptors

Primer	Sequence (5' to 3')
IGF-I (Accession # X15726)	
Forward	TGTGATTTCTTGAAGCAGGTGAA
Reverse	AGCACAGGGCCAGATAGAAGAG
TaqMan probe	6FAM-GCCCATCACATCCTCCTCGCA-TAMRA
$\beta_1$ -adrenergic receptor (Accession # AF188187)	
Forward	GTGGGACCGCTGGGAGTAT
Reverse	TGACACACAGGGTCTCAATGC
TaqMan probe	6FAM-CTCCTTCTTCTGCGAGCTCTGGACCTC-
$\beta_2$ -adrenergic receptor (Accession # NM_174231)	
Forward	CAGCTCCAGAAGATCGACAAATC
Reverse	CTGCTCCACTTGACTGACGTTT
TaqMan probe	6FAM-AGGGCCGCTTCCATGCCC-TAMRA
$\beta_3$ -adrenergic receptor (Accession # X85961)	
Forward	AGGCAACCTGCTGGTAATCG
Reverse	GTCACGAACACGTTGGTCATG
TaqMan probe	6FAM-CCCGGACGCCGAGACTCCAG-TAMRA

**Table 3.3** Effects of steroidal implantation and feeding ractopamine on nitrogen retention and diet digestibility in growing steers

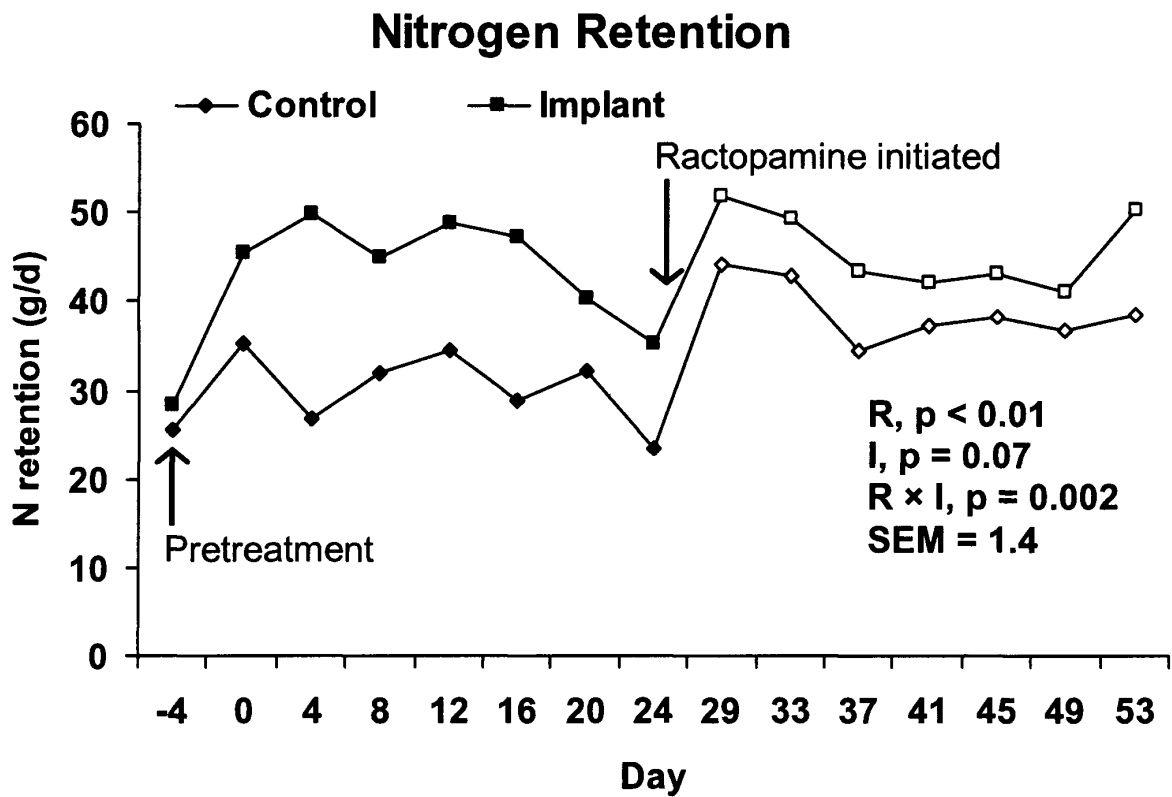
Item	No ractopamine		Ractopamine-HCl, 200 mg/day		SEM	p-value		
	Control	Implant	Control	Implant		Implant	Ractopamine	I × R
Nitrogen, (g/day)								
Dietary intake	143.2	140.4	139.6	135.6	3.9	0.65	< 0.001	0.42
Faecal	35.2	35.4	29.1	29.9	2.9	0.93	< 0.001	0.73
Urinary	77.2 <sup>z</sup>	60.8 <sup>x</sup>	71.2 <sup>y</sup>	60.2 <sup>x</sup>	4.0	0.07	0.003	0.02
Ammonia	6.9 <sup>y</sup>	3.1 <sup>x</sup>	4.7 <sup>x</sup>	4.1 <sup>xy</sup>	1.2	0.40	0.31	0.006
Urea	63.2	49.9	60.6	46.7	4.3	0.19	0.02	0.81
Retained	30.4 <sup>x</sup>	44.6 <sup>z</sup>	39.0 <sup>y</sup>	45.9 <sup>z</sup>	1.4	0.07	< 0.001	0.002
Dry matter digestibility (%)	76.9	77.7	79.0	80.2	1.9	0.61	0.0002	0.75

Each response represents an average over 28 days.

Means values in rows not bearing a common superscript letter differ,  $p < 0.05$ .

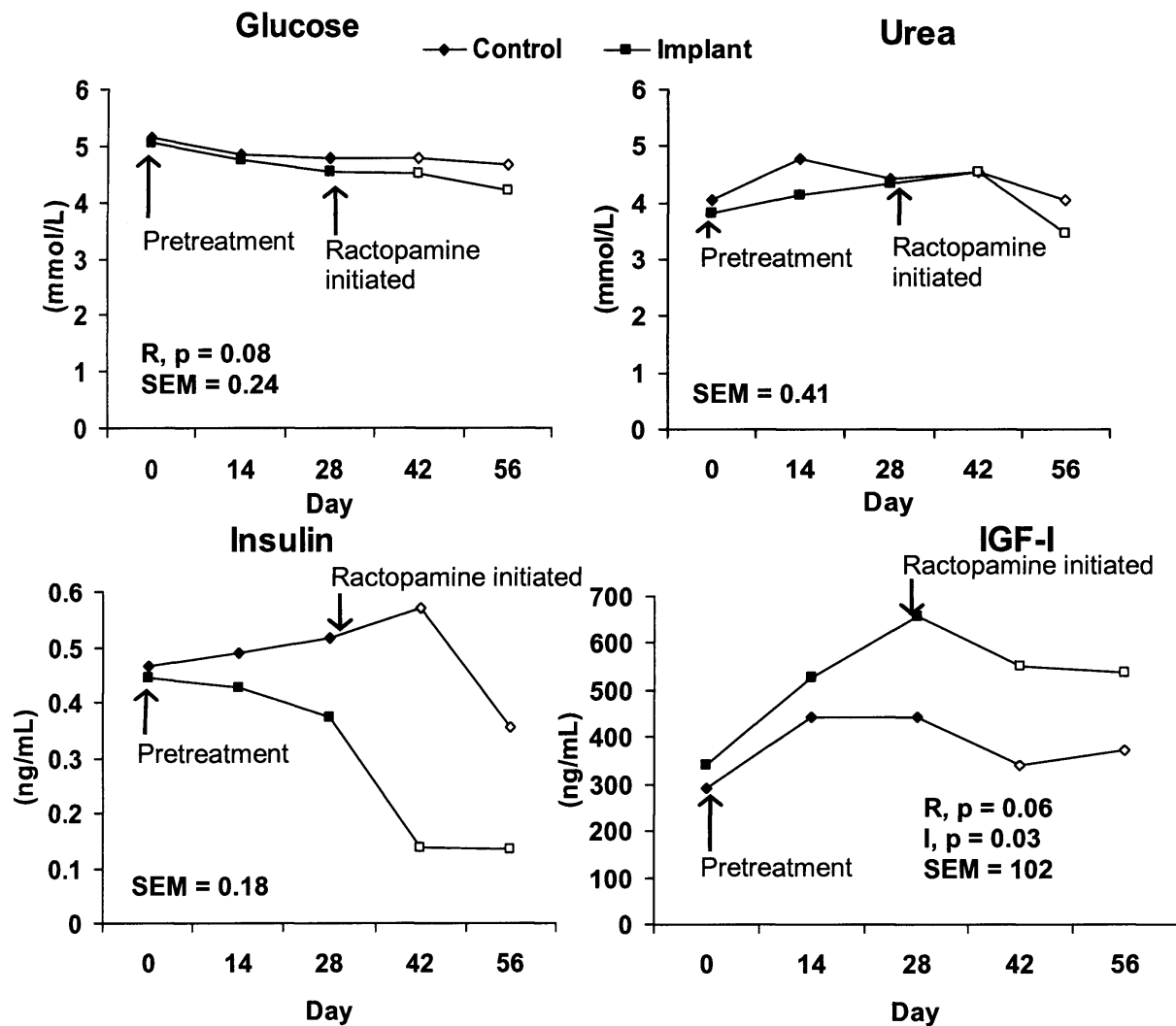
**Table 3.4** Effects of steroidal implantation on mRNA expression in M. semimembranosus and liver of ractopamine-treated steers

Item	Control	Implant	SEM	p-value
M. semimembranosus, mRNA	(Arbitrary Units)			
β <sub>1</sub> adrenergic receptor	47	23	13	0.32
β <sub>2</sub> adrenergic receptor	16 305	29 246	8965	0.08
β <sub>3</sub> adrenergic receptor	78	24	34	0.36
IGF-I	956	3288	792	0.11
Liver, mRNA				
IGF-I	37 252	78 554	19 536	0.16



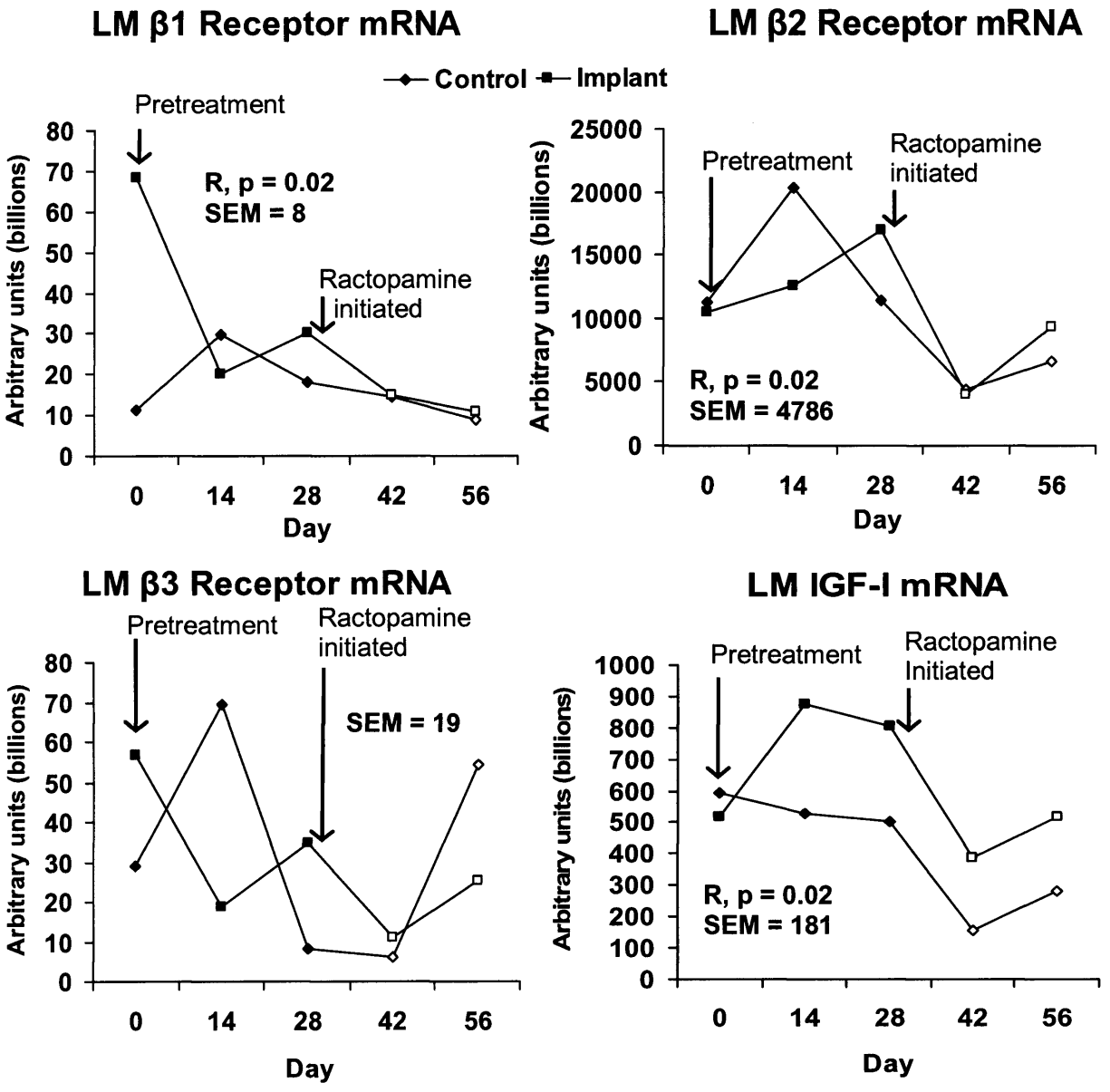
**Figure 3.1** Effects of implantation and ractopamine on nitrogen retention over time in Holstein steers.

Each value represents nitrogen retention over a 4-day period (n=3). Days listed represent first day of collection. Filled symbols represent time-points without ractopamine, whereas open symbols represent times-points when ractopamine was feed. R = Ractopamine, I = Implant.



**Figure 3.2** Effects of implantation and ractopamine on blood metabolites over time in Holstein steers.

For each point,  $n=3$ . Filled symbols represent time-points without ractopamine, whereas open symbols represent times when ractopamine was fed. Blood samples were collected 2 h after the morning feeding. R = Ractopamine, I = Implant.



**Figure 3.3** Effects of implantation and ractopamine on *M. longissimus* mRNA expression over time in Holstein steers.

For each point, n=3. Filled symbols represent time-points without ractopamine, whereas open symbols represent times when ractopamine was fed. R = Ractopamine.

# **CHAPTER 4 - Effects of Ractopamine on Growth-related Criteria in Finishing Steers and Heifers**

D. K. Walker, E. C. Titgemeyer, T. J. Baxa, K. Y. Chung, D. E. Johnson, S. B. Laudert,  
and B. J. Johnson



## Abstract

Our experiment evaluated growth-related responses to ractopamine in steers and heifers. Sixteen Angus steers and 16 Angus heifers (493 kg) housed in individual pens were used in a randomized complete block design. At 90 to 97 d prior to the experiment, steers were implanted with 120 mg trenbolone acetate and 24 mg estradiol-17 $\beta$  (Component TE-S) and heifers were implanted with 140 mg trenbolone acetate and 14 mg estradiol-17 $\beta$  (Component TE-H). Treatments were arranged as a 2  $\times$  2 factorial and included: gender (steer vs. heifer) and ractopamine-HCl (0 or 200 mg/d) for the final 28 d. Cattle were fed a diet based on steam-flaked corn once daily. Blood and longissimus (LM) and biceps femoris (BF) biopsy samples were collected on d 0 (prior to ractopamine feeding) and after 14 and 28 d of ractopamine feeding. Serum insulin-like growth factor (IGF)-I concentrations were greater in steers than heifers ( $P < 0.001$ ), and steers demonstrated greater IGF-I mRNA expression in BF than heifers ( $P = 0.05$ ). In vitro protein synthesis rates tended to be greater in LM of steers than in LM of heifers ( $P = 0.10$ ). Ractopamine decreased serum IGF-I concentrations in heifers on d 14, but increased serum IGF-I concentrations in steers on d 28 (gender  $\times$  ractopamine  $\times$  day interaction;  $P = 0.03$ ). Ractopamine did not affect mRNA expression of IGF-I in BF ( $P = 0.21$ ) or in LM ( $P = 0.22$ ). Expression of IGFBP-3 and -5 mRNA in LM and BF were not affected by ractopamine ( $P \geq 0.42$ ). Ractopamine decreased in vitro protein degradation rates ( $P = 0.03$ ) and numerically increased calpastatin mRNA expression in LM ( $P = 0.23$ ), but numerically increased protein degradation rates in BF ( $P = 0.19$ ). Ractopamine decreased myosin heavy chain IIA mRNA expression in BF ( $P = 0.04$ ). Ractopamine decreased  $\beta_2$ -receptor mRNA expression in LM of steers on d 14, but numerically increased it in steers on d 28; in contrast expression of  $\beta_2$ -receptor mRNA in LM of heifers was not affected by ractopamine (gender  $\times$  ractopamine  $\times$  day interaction;  $P = 0.03$ ). The effects of ractopamine on protein degradation seem to be somewhat different between LM and BF. Ractopamine does not appear to greatly impact muscle expression of mRNA for IGF-I or for the IGFBP-3 and -5.

## **Introduction**

Ractopamine-HCl is a  $\beta$ -adrenergic agonist that is approved for use in finishing cattle. Ractopamine administration elicits responses through  $\beta$ -adrenergic receptors ( $\beta$ AR;  $\beta_1$  and  $\beta_2$ ) and results in increased muscle mass with minimal effects on adipose tissue (Mersmann, 1998). Ractopamine-HCl fed at 200 mg/d for the final 28 d can improve ADG and G:F by 17 and 18% (Laudert et al., 2004) and by 15 and 17% (Gruber et al., 2007) in finishing steers and by 18 and 15% (Schroeder et al., 2005b) and by 11 and 12% (Laudert et al., 2007) in finishing heifers. In contrast, Quinn et al. (2006) reported no change in carcass gain, DMI, and carcass efficiency when non-implanted heifers were fed 200 mg/d ractopamine-HCl for the final 28 d. Sissom et al. (2007) demonstrated 2.2 and 4.0% improvements in ADG and G:F with no effect on DMI over the entire 178 to 187 d feeding period in finishing heifers fed 200 mg/d ractopamine-HCl for the final 28 d. Hot carcass weight has been increased by 8 (Winterholler et al., 2007), 6 (Laudert et al., 2004), and 5.5 kg (Gruber et al., 2007) in feedlot steers. In feedlot heifers, HCW was increased by 4.6 (Laudert et al., 2007) and 2.9 kg (Schroeder et al., 2005b).

Although, improvements in growth and carcass performance are observed in feedlot steers and heifers, the responses in heifers seem more variable, suggesting that ractopamine may be acting somewhat differently in heifers than in steers. Our objectives were to determine the effects of feeding ractopamine to finishing steers and heifers on skeletal muscle protein turnover and gene expression, and to determine if responses differed between steers and heifers.

## **Materials and Methods**

Procedures for this study were approved by the Kansas State University Institutional Animal Care and Use Committee.

### ***Animals***

Sixteen Angus steers and 16 Angus heifers were used in a randomized complete block design experiment to evaluate the responses of steers and heifers to ractopamine feeding. Cattle originated from 2 different sires and were fed similarly for 1 mo prior to arrival. Cattle were received 100 to 107 d prior to initiation of the experiment, grouped by gender in 2 pens, and transitioned from a 60% concentrate:40% roughage diet to a 90% concentrate:10% roughage diet

within the first 30 d. Thereafter, all cattle were fed a common diet based on steam-flaked corn for ad libitum consumption for 70 to 77 d prior to initiation of the trial. Ten days after arrival, corresponding to 90 to 97 d prior to cattle starting on the trial, steers were implanted with 120 mg TBA and 24 mg E<sub>2</sub> (Component TE-S, Vet Life, West Des Moines, IA) and heifers were implanted with 140 mg TBA and 14 mg E<sub>2</sub> (Component TE-H, Vet Life). Steers and heifers averaged 15.6 and 15.3 mo of age, respectively, when started on trial, and as a group the cattle graded 68% USDA Choice and 45% USDA Yield grade 2 and 50% USDA Yield grade 3.

### ***Overall Design***

Treatments included gender (steer or heifer) and ractopamine-HCl (0 or 200 mg/d; Optaflexx; Elanco Animal Health, Greenfield, IN). Steers and heifers were blocked (8 blocks with each containing 2 steers and 2 heifers) based on BW and ADG during the 90 d prior to the start of the experiment. To accomplish blocking, cattle were sorted into the heaviest 8 and the lightest 8 animals within each gender. Within gender and BW group, cattle were sorted into groups of 2 animals based on ADG. Groups of 4 pens were assigned to blocks. Within each block of 4 pens, treatments were randomly assigned to pens. Cattle within each block were then assigned to treatment. The gender was predetermined, so only the ractopamine treatment was randomized between the 2 cattle of the same gender within each block, and this was accomplished by the flip of a coin. The cattle assigned to each treatment were then placed in the appropriate pen within their block. All cattle were fed and housed individually in 4.5 × 1.5 m pens for 7 to 8 d prior to initiation of the experiment and were given ad libitum access to water and feed (Table 4.1) for 28 d, which represents both the shortest feeding time for which ractopamine usage is labeled as well as the most typical feeding period for ractopamine by the feedlot industry. Cattle were fed once daily at 1600 with bunk management designed to yield slick bunks at feeding.

Due to the number of cattle and logistics of the procedures imposed, complete blocks of steers and heifers were started on trial staggered over time. Trial initiation for blocks 1 and 2 occurred 1 d prior to that for blocks 3 and 4, 7 d prior to that for blocks 5 and 6, and 8 d prior to that for blocks 7 and 8. Beginning at 0630 on each sampling day, a portable chute was placed in front of each pen and cattle were placed into the chute. Cattle were bled, and biopsy samples were collected from the biceps femoris (BF) and LM. Sample collection occurred on d 0 before initiation of ractopamine feeding, and on d 14 and 28 of ractopamine feeding. After sample

collection on d 0 (before initiation of ractopamine feeding) and on d 28 of ractopamine feeding for each block, cattle were weighed at 1100. For 2 blocks, cattle assigned to the ractopamine treatment mistakenly received ractopamine on the day before the initial sampling; their d-0 data were not used for statistical analysis (see below), and samples collected after 15 and 29 d of ractopamine feeding were considered equivalent to those collected after 14 and 28 d of ractopamine feeding.

### ***Blood Samples***

Jugular blood samples were collected into vacuum tubes (Becton Dickinson, Franklin Lakes, NJ) containing sodium heparin, immediately placed on ice, and centrifuged for 20 min at  $1,000 \times g$  to obtain plasma. Blood was also collected into vacuum tubes without additives, allowed to clot for 24 h at 4°C, and then centrifuged for 20 min at  $1,000 \times g$  to obtain serum. Plasma samples were stored (-20°C) for later analysis of glucose (Gochman and Schmitz, 1972) and urea (Marsh et al., 1965). Sera were stored (-20°C) for later analysis of insulin (RIA kit, DSL-1600; Diagnostic Systems Laboratories, Webster, TX; intra-assay CV = 4.7%, assay sensitivity = 0.00838 ng/mL; Greenwood et al., 2001) and insulin-like growth factor (IGF)-I (IGF-I coated-tube immunoradiometric assay kit, DSL-5600; Diagnostic Systems Laboratories; intra-assay CV = 4.3%, assay sensitivity = 5.0 ng/mL; Greenwood et al., 2001).

### ***Muscle Biopsies***

Biopsy samples were collected from the BF and LM (Dunn et al., 2003; Pampusch et al., 2003) for measuring in vitro protein synthesis and degradation and gene expression. Biopsies on d 0 and 28 were collected from the left side and on d 14 from the right side, with LM sampling sites being initially at the last rib and moving 5 cm anterior to the previous site and with BF sampling sites being initially mid-way between the trochanter major of the femur and the tuber ischii and moving 5 cm ventral to the previous site.

### ***In Vitro Protein Synthesis***

In vitro protein synthesis rates were determined as described by Greig et al. (1986) from biopsy samples collected on d 0, 14, and 28 from the BF and LM. Samples (150 to 300 mg) were blotted on sterile gauze and placed in pre-incubation medium for transport to the lab. The pre-incubation media contained Krebs-Ringer bicarbonate buffer, saturated with 95% O<sub>2</sub>:5% CO<sub>2</sub> by bubbling for 30 min, 10 mM glucose, 0.1 IU porcine insulin/mL, and 20 AA (mM; Ala 4.5, Arg 2.0, Asn 0.7, Asp 0.35, Cys 0.7, Glu 2.0, Gln 3.5, Gly 4.0, His 0.8, Ile 1.0, Leu 1.7, Lys

4.0, Met 0.7, Phe 0.8, Pro 1.8, Ser 2.8, Thr 3.0, Trp 0.7, Tyr 0.7, and Val 2.0). For each muscle within each animal, 20 to 40 mg of tissue were placed into each of 5 incubation tubes containing 3.0 mL of incubation medium, re-gassed with 95% O<sub>2</sub>:5% CO<sub>2</sub>, and incubated for 3 h at 37°C in a metabolic shaker bath. The incubation media contained the pre-incubation media plus 1.8 µCi L-[<sup>3</sup>H]-tyrosine. Following incubation, tissue samples were removed from the media, blotted, weighed, frozen in liquid N<sub>2</sub>, and stored at -20°C. Media were frozen in liquid N<sub>2</sub> and stored at -20°C.

Muscle samples were homogenized (Tissue Tearor; Biospec Products, Inc., Bartlesville, OK) in 2.5 mL of 10 mM potassium phosphate buffer (pH 7.4 at 0 to 5°C). Protein was precipitated from 2.0 mL of homogenate with 1 mL of 30% (wt/vol) trichloroacetic acid (TCA) at 0 to 5°C and centrifuged at 11,000 × g for 10 min. The protein pellet was washed twice with 10% (wt/vol) TCA and dissolved in 1.0 mL of tissue solubilizer (NCS-II Tissue Solubilizer, Amersham Biosciences, Little Chalfont Buckinghamshire, England) at 65°C for 30 min. The solution was poured into a scintillation vial with 15 mL of scintillation cocktail (ScintiSafe Plus 50%, Fisher Scientific, Hanover Park, IL). Samples were allowed to stand overnight in low lighting to reduce chemiluminescence before being counted in a scintillation counter. The medium (3.0 mL) was acidified with 1.0 mL of 40% TCA (wt/vol) at 0 to 5°C and centrifuged at 11,000 × g for 10 min. An aliquot of the supernatant was analyzed for tyrosine concentration as described by Waalkes and Udenfriend (1957), and a 1.0-mL aliquot was counted for radioactivity.

Protein synthesis rates were estimated by <sup>3</sup>H-Tyr incorporation into protein. The equation utilized to calculate synthesis rates was: protein synthesis rate = [dpm in protein / specific activity of free Tyr, dpm/µg] / (wet tissue weight, g × 3 h). Concentrations of Tyr (0.7 mM) were 10 times that of bovine plasma and, therefore, Tyr was assumed to equilibrate between the medium and intracellular precursor pool. Consequently, Tyr specific activity in the medium was used as the specific activity of the precursor pool. The median value from the 5 replicates was used for statistical analysis.

### ***In Vitro Protein Degradation***

In vitro protein degradation rates were determined as described by Greig et al. (1986) from biopsy samples collected on d 0, 14, and 28 from the BF and LM. Samples (150 to 300 mg) were blotted on sterile gauze and placed in pre-incubation medium for transport to the lab.

The pre-incubation medium contained Krebs-Ringer bicarbonate buffer with 10 mM glucose. For each muscle within each animal, 20 to 40 mg of tissue were placed into each of 6 incubation tubes containing 3.0 mL of incubation medium (pre-incubation media and 0.5 mM cycloheximide) and re-gassed with 95% O<sub>2</sub>:5% CO<sub>2</sub>. Three incubation tubes were immediately frozen in liquid N<sub>2</sub> and were used for measuring background concentrations of free Tyr. Three tubes were incubated for 3 h at 37°C in a metabolic shaker bath. Following incubation, samples were frozen in the media in liquid N<sub>2</sub> and stored at -20°C.

Muscle samples were homogenized in medium. An aliquot of the homogenate was assayed for protein concentration via Coomassie Blue Protein Assay Kit (Pierce, Rockford, IL). Protein was precipitated from 1.0 mL of homogenate with 0.5 mL of 30% TCA (wt/vol) at 0 to 5°C and centrifuged at 11,000 × g for 10 min. A 1.0-mL aliquot of the supernatant was analyzed for Tyr concentration (Waalkes and Udenfriend, 1957).

Protein degradation rates were estimated by Tyr release. Cycloheximide was included in the incubation media to inhibit protein synthesis, thereby preventing re-utilization of released Tyr. The equation utilized to calculate degradation rates was : protein degradation rate = [(Tyr in incubated samples/(mg protein × 0.025)) – (Tyr in un-incubated samples/(mg protein × 0.025))] / 3 h, where 0.025 is the fraction of skeletal muscle as Tyr (Lobley et al., 1980) and 3 h is the incubation time.

### ***RNA Isolation***

Muscle biopsy samples (2 g) were rapidly frozen in liquid N<sub>2</sub> and stored at -80°C for subsequent RNA isolation. Total RNA was isolated from BF and LM samples as described by Dunn et al. (2003) and Pampusch et al. (2003). The concentration of RNA was determined by absorbance at 260 nm. To verify the integrity of the RNA, visualization of the 28S and 18S ribosomal RNA (rRNA) was accomplished with a 2100 Bioanalyzer (Agilent, Foster City, CA). One microgram of total RNA was reverse-transcribed to produce the first-strand complementary DNA (cDNA) using TaqMan reverse transcriptase (Applied Biosystems, Foster City, CA) following the protocol recommended by the manufacturer. Random hexamers were used as primers in cDNA synthesis.

### ***Real Time PCR***

Real-time quantitative-PCR was used to measure the quantity of mRNA for β<sub>1</sub>-, β<sub>2</sub>-, and β<sub>3</sub>-adrenergic receptors, IGF-I (Class 1), IGFBP-3 and -5, calpastatin, myosin heavy chain

(MHC) IIA, and 18S rRNA in total RNA isolated from BF and LM. Measurement of the relative quantity of cDNA was conducted using TaqMan Universal PCR Master Mix (Applied Biosystems), 900 nM of the appropriate forward and reverse primers, 200 nM of the appropriate TaqMan detection probe, and 1  $\mu$ L of the cDNA mixture. Sequences for primers and probes are presented in Table 4.2. The primers for IGF-I spanned exon 1 and exon 3 and were designed to measure Class 1 IGF-I mRNA. Commercially available eukaryotic 18S rRNA primers and probes were used as an endogenous control (Applied Biosystems; Genbank Accession no. X03205). Assays were performed in an ABI Prism 7000 sequence detection system (Applied Biosystems) using thermal cycling parameters recommended by the manufacturer (40 cycles of 15 sec at 95°C and 1 min at 60°C). Relative expressions of mRNA for  $\beta_1$ -,  $\beta_2$ -, and  $\beta_3$ -adrenergic receptors, IGF-I (Class 1), IGFBP-3 and -5, calpastatin, and MHC IIA were normalized to the 18S rRNA endogenous control and expressed in arbitrary units. Samples with a 18s rRNA value for thermal cycles below 10 and above 13 were re-run using newly synthesized cDNA for the PCR reactions. Any samples associated with a gene arbitrary unit value grossly outside the typical range were re-run using newly synthesized cDNA for the PCR reactions. For any re-run samples remaining outside the aforementioned ranges, RNA was re-isolated, cDNA synthesized, and the PCR reaction was re-run. One sample from d 0 was misplaced and, therefore, the data were missing.

#### ***DNA, RNA, and Protein Concentrations***

Concentrations of DNA, RNA, and protein were determined in LM and BF biopsy samples collected on d 28. Approximately 300 mg of tissue was ground into powder using a mortar and pestle submerged in liquid N<sub>2</sub>. One hundred milligrams of ground sample, in duplicate, was used to isolate RNA as described previously. Concentration of RNA was determined by absorbance at 260 nm, and the highest value of duplicate samples was used.

Twenty five milligrams of ground sample, in duplicate, were used to isolate DNA via DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA; Blin and Stafford, 1976). To determine DNA concentration, 100  $\mu$ L of sample was mixed with phosphate buffer containing bisbenzimidazole (compound H 33258) to a final concentration of 1  $\mu$ g/mL as described by Labarca and Paigen (1980). Fluorescence was measured with a fluorimeter (HP 1046A Programmable Fluorescence Detector; Santa Clara, CA; excitation, 356 nm; emission, 458 nm) within 16 h of mixing. The concentrations of DNA were determined by comparison to an

external standard curve of bovine calf thymus DNA in a range to 5.0  $\mu\text{g}/\text{mL}$ . The highest value of the duplicate samples was used. For one heifer fed ractopamine for LM, analysis of muscle composition yielded unreasonable data, and therefore observations for this sample were excluded from statistical analysis.

Ten milligrams of ground sample was placed into 500  $\mu\text{L}$  of phosphate buffer (0.05  $M$   $\text{Na}_2\text{HPO}_4$ , 2  $M$   $\text{NaCl}$ , adjusted to pH 7.4) and homogenized with a polytron homogenizer. Protein concentrations were determined from an aliquot of the homogenate using the Coomassie Blue Protein Assay Kit (Pierce).

### ***Statistical Analysis***

Data were analyzed using the MIXED procedure of SAS System for Windows Release 9.1 (SAS Inst. Inc., Cary, NC). Performance and RNA, DNA, and protein concentration data were analyzed as a randomized complete block design. The model contained the effects gender, ractopamine, and ractopamine  $\times$  gender. Block was included as a random effect. All other data were analyzed as a randomized complete block design with repeated measures with d-0 values used as a covariate. The model contained the effects of the covariate, gender, ractopamine, ractopamine  $\times$  gender, day, ractopamine  $\times$  day, gender  $\times$  day, and ractopamine  $\times$  gender  $\times$  day. Block was included as a random effect. The repeated statement included day as the repeated variable with animal as experimental unit and the covariance structure as unstructured. To estimate missing values from d 0, values were predicted using the same model but without the covariate. Because the covariates (d 0 values) would remove most of the effects of gender, which was fixed before the d 0 measures, data were analyzed for the main effect of gender without the use of covariates as well as without inclusion of predicted d 0 values. Treatment means were computed using the LSMEANS option, and pair-wise  $t$ -tests were used to separate means when interactions were significant.

## **Results and Discussion**

### ***Effects of Gender and Ractopamine on Performance***

Performance data for steers and heifers are presented in Table 4.3. Heifers fed ractopamine consumed 16% less DM than control heifers, but in steers there were no differences in DMI in response to ractopamine (gender  $\times$  ractopamine interaction;  $P = 0.05$ ). Dry matter intake in finishing heifers generally is not affected by ractopamine (Chapter 2 of this thesis;



Schroeder et al., 2005b; Sissom et al., 2007). Gruber et al. (2007) observed no change in DMI in feedlot steers administered ractopamine. Heifers assigned to the ractopamine treatment consumed less feed relative to the other cattle for the week prior to the study (7.7, 6.3, 6.7, and 7.2 kg for control heifers, heifers fed ractopamine, control steers, and steers fed ractopamine), suggesting that the low DMI for ractopamine-fed heifers probably does not reflect a response to ractopamine.

The BW of heifers on d 0 and 28 were less than the steers ( $P < 0.001$ ). The final BW (d 28) for steers and heifers were not affected by ractopamine feeding. Daily gains and G:F were not affected by ractopamine or gender, although ractopamine numerically increased ADG (16%) and G:F (21%) in steers. Our ability to detect performance responses to ractopamine was limited by the unexpectedly low DMI of heifers fed ractopamine. Gain efficiencies above maintenance were calculated (Table 4.3) because they are independent of DMI, and ractopamine tended ( $P = 0.07$ ) to increase G:F above maintenance by 20%, which is similar to improvements in G:F observed in other trials. Schroeder et al. (2005a) demonstrated 20 and 21% increases in ADG and G:F in feedlot steers fed 20 ppm ractopamine for the final 28 to 42 d. Gruber et al. (2007) reported 15 and 17% improvements in ADG and G:F in implanted finishing steers when fed 200 mg/d ractopamine for the final 28 d. Schroeder et al. (2005b) observed increases of 18 and 15% in ADG and G:F in finishing heifers fed 20 ppm ractopamine for the final 28 to 42 d. Laudert et al. (2007) demonstrated 11 and 14% increases in ADG and G:F in implanted finishing heifers fed 200 mg/d ractopamine for the final 28 d. Implanted finishing heifers in response to feeding 200 mg/d ractopamine for the final 28 d demonstrated 18% increases in ADG and G:F (Chapter 2 in this thesis).

### ***Effect of Gender***

Data for blood metabolites are presented in Table 4.4. Steers tended to have greater plasma glucose concentrations than heifers ( $P = 0.08$ ). Plasma urea concentrations in steers were less ( $P = 0.003$ ) than those in heifers. Serum insulin concentrations were not affected by gender. Serum IGF-I concentrations were greater ( $P < 0.001$ ) in steers than heifers. Plouzek and Trenkle (1991) using non-implanted cattle demonstrated 20 and 43% greater serum IGF-I concentrations in steers than in heifers at 12 and 15 mo of age. Because steers deposit more lean tissue than heifers at the end of the finishing period, lower plasma urea and greater serum IGF-I concentrations might be expected in steers compared to heifers.

Protein synthesis and degradation rates are presented in Table 4.4. In LM, protein synthesis rates tended to be higher in steers compared to heifers ( $P = 0.10$ ). Protein synthesis rates in BF ( $P = 0.76$ ) and protein degradation rates in BF and LM ( $P \geq 0.42$ ) were not affected by gender.

Data for mRNA expression of IGF-I, IGFBP-3 and -5, MHC IIA, calpastatin, and  $\beta_1$ -,  $\beta_2$ -, and  $\beta_3$ - receptors in BF and LM are presented in Table 4.4. In BF, steers demonstrated greater IGF-I mRNA expression than heifers ( $P = 0.05$ ). Steers demonstrated numerically greater mRNA expression of IGFBP-3 ( $P = 0.10$ ) and -5 ( $P = 0.17$ ) in LM compared to heifers. Calpastatin and MHC IIA mRNA expression in BF ( $P = 0.59$ ) and LM ( $P = 0.22$ ) were not affected by gender.  $\beta_1$ - and  $\beta_2$ -receptor mRNA expression in BF and  $\beta_1$ - and  $\beta_3$ -receptor mRNA expression in LM were not affected by gender.  $\beta_3$ -receptor mRNA expression in BF ( $P = 0.08$ ) and  $\beta_2$ -receptor mRNA expression in LM ( $P = 0.11$ ) tended to be greater in steers compared to heifers. Greater amounts of  $\beta_2$ -receptor mRNA may allow for steers to respond better than heifers to  $\beta$ -agonists such as ractopamine.

Data for RNA, DNA, and protein concentrations and protein:DNA, protein:RNA, and RNA:DNA ratios are presented in Table 4.7. No effect of gender on RNA, DNA, and protein concentration were observed. Additionally, protein:DNA, protein:RNA, and RNA:DNA ratios were not affected by gender.

As expected, steers demonstrated differences from heifers that are characteristic of greater lean growth such as lower plasma urea and greater serum IGF-I concentrations, in addition to numerically greater protein synthesis rates in the LM.

### ***Effects of Ractopamine and Interaction of Ractopamine with Gender***

Data for blood metabolites are presented in Tables 4.5 and 4.6. Plasma glucose concentrations were not affected by ractopamine. Ractopamine tended to decrease plasma urea concentrations ( $P = 0.06$ ). Growing Holstein steers demonstrated no change in plasma urea concentrations in response to ractopamine (Chapter 3 in this thesis). In contrast, Dunshea and King (1994) reported a 5% decrease in plasma urea-N in gilts fed 20 ppm ractopamine for 22 d. Ractopamine increases lean tissue deposition and increases N retention (Chapter 3 of this thesis) and, therefore, would be expected to decrease urea production. Serum insulin concentrations were not affected by ractopamine. Similarly, growing Holstein steers demonstrated no change in insulin concentrations when fed ractopamine for 28 d (Chapter 3 of this thesis). In response to

ractopamine feeding, serum IGF-I concentrations (Figure 4.1) were decreased in heifers on d 14, not different in steers on d 14 and in heifers on d 28, but increased in steers on d 28 (gender  $\times$  ractopamine  $\times$  day interaction;  $P = 0.03$ ). The serum IGF-I response in steers fed ractopamine might allow an extended growth curve enabling them to deposit more lean tissue compared to heifers. Heifers fed ractopamine had lower DMI and it is plausible that the lower DMI, independent of ractopamine, resulted in decreases in circulating IGF-I concentrations. Ellenberger et al. (1989) demonstrated decreases in serum IGF-I concentrations when steers were feed restricted. The majority of IGF-I in circulation is derived from hepatic production and, therefore, changes in serum IGF-I concentrations in steers and heifers fed ractopamine could be attributed to ractopamine affecting hepatic IGF-I synthesis or release, or ractopamine might alter the IGF-BP such that IGF-I clearance from the blood is affected. Beermann et al. (1987) reported 46.5 and 21.5% decreases in serum IGF-I concentrations in lambs fed cimaterol for 6 and 12 wk compared to lambs not fed cimaterol. Serum IGF-I concentrations tended to decrease by 13% in Holstein steers in response to feeding 200 mg/d ractopamine for 28 d (Chapter 3 in this thesis).

In vitro skeletal muscle protein synthesis and degradation were measured as an evaluation of skeletal muscle protein metabolism. Protein synthesis and degradation rates are presented in Tables 4.5 and 4.6. Protein synthesis rates were not affected by ractopamine. Ractopamine decreased in vitro protein degradation rates in LM ( $P = 0.03$ ), but numerically increased them in BF ( $P = 0.19$ ). Wheeler and Koohmaraie (1992) demonstrated a 27% decrease in fractional protein degradation rates of skeletal muscle myofibrillar protein and a numerical increase in fraction protein synthesis rates of skeletal muscle myofibrillar protein after 3 wk in steers fed 3 ppm of L<sub>644,969</sub>. Bergen et al. (1989) reported that fraction protein synthesis and degradation rates were 4.4%/d and 3.4%/d for control and 6.1%/d and 4.9%/d in the semitendinosus of pigs fed 20 ppm ractopamine for 21 to 35 d. Ractopamine decreased rates of protein degradation in the LM of steers and heifers in support of the data reported by Wheeler and Koohmaraie (1992), but this contrasts data reported by Bergen et al. (1989). That protein degradation rates in the BF were numerically increased by ractopamine suggests a difference between muscles in response to ractopamine feeding. Although rates of protein synthesis and degradation will not be reflective of in vivo rates due to the nature of the sample and assay, our goal was to assess changes due to ractopamine. Our samples were collected using a Bergstrom biopsy needle that produced small tissue pieces, and this could limit our ability to measure true protein synthesis and degradation

rates. Additionally, our in vitro protein synthesis and degradation assays did not differentiate the myofibrillar protein fraction from total protein and, therefore, differences may not reflect changes in myofibrillar protein turnover.

Data for mRNA expression of IGF-I, IGFBP-3 and -5, MHC IIA, calpastatin, and  $\beta_1$ -,  $\beta_2$ -, and  $\beta_3$ -receptors in BF and LM are presented in Tables 4.5 and 4.6. Ractopamine did not significantly alter mRNA expression of IGF-I in BF ( $P = 0.21$ ) and in LM ( $P = 0.22$ ), although in both muscles abundance of IGF-I mRNA was numerically increased. In LM, mRNA expression of IGF-I decreased in growing Holstein steers fed 200 mg/d ractopamine for 14 or 28 d (Chapter 3 in this thesis). Sissom et al. (2007) demonstrated a reduction of IGF-I mRNA expression in response to ractopamine in semimembranosus of heifers initially implanted with 80 mg TBA/8 mg E<sub>2</sub> and re-implanted with 200 mg TBA 96 d prior to feeding 200 mg/d ractopamine for the final 28 d. However, in the same study, heifers implanted with 200 mg TBA/20 mg E<sub>2</sub> 154 d prior to ractopamine feeding demonstrated a numerical increase in IGF-I mRNA expression in the semimembranosus, suggesting that the intensity of the implant program may affect the responsiveness of heifers to ractopamine. Yimlamai et al. (2005) reported that clenbuterol fed to rats for 2 wk decreased protein expression of IGF-I in the plantaris muscle (fast twitch), but did not affect protein expression of IGF-I in the tibialis anterior muscle. In contrast, Awede et al. (2002) demonstrated a 5-fold increase in IGF-I mRNA expression in the soleus muscle (slow twitch) of 3 mo old rats administered 10 ppm clenbuterol in the drinking water for 3 d. Fiber composition of the muscle might determine responsiveness to  $\beta$ -agonists, but in our study, IGF-I mRNA expression numerically increased in both BF and LM due to the ractopamine. Clearly, the role of local IGF-I expression in response to  $\beta$ -agonists is still equivocal.

The IGFBP regulate activity and clearance of IGF-I. The mRNA expression of IGFBP-3 and -5 were not affected in LM or BF by ractopamine ( $P \geq 0.42$ ). In contrast, Awede et al. (2002) demonstrated a 5-fold increase in IGFBP-5 mRNA expression in the soleus muscle of 3 mo old rats administered clenbuterol for 3 d. The biological activity of IGF-I is regulated by 1 of 6 IGFBP. A majority of IGF-I in circulation is bound to IGFBP, which can either inhibit or facilitate IGF-I binding to the IGF-I receptor (Duan, 2002). The most abundant IGFBP in circulation is IGFBP-3, and IGFBP-3 and -5 have been implicated in inhibition of cell proliferation via IGF-I independent actions that negatively impact muscle growth (Kamango-Sollo et al., 2005; Yang et al., 1999). The lack of effect of ractopamine on mRNA expression of

IGFBP-3 and -5 in LM and BF suggest that, if ractopamine impacts IGFBP, it does so through effects on tissues other than muscle.

Ractopamine decreased MHC IIA mRNA expression in BF ( $P = 0.04$ ), but not in LM ( $P = 0.99$ ). Vestergaard et al. (1994) reported a decrease in frequency of type IIA fibers from 24.2 to 8.6% in LM and from 24.3 to 6.7% in semitendinosus of Friesian bulls fed 0.06 ppm cimaterol for 90 d, whereas frequency of type IIB increased from 51.8 to 71.1% in LM and from 61.8 to 81.6% in the semitendinosus. Miller et al. (1988) demonstrated a 19% increase in type II fiber diameter with no change in type I diameter and frequency of type I and II in the LM of heifers fed 10 mg/d clenbuterol for 50 d. Depreux et al. (2002) demonstrated a decrease in MHC IIA mRNA expression in LM and semimembranosus of pigs fed 20 ppm ractopamine for 28 d. These data demonstrate that administration of  $\beta$ -agonists can either convert slow twitch fibers to fast twitch fibers as demonstrated by changes in distribution and in gene expression of the MHC isoforms or alter protein turnover via changes in fiber diameter and in gene expression of the MHC isoforms. Our MHC IIA data could suggest that ractopamine induced alterations in fiber type in BF, but not in LM. It is also possible that decreases in MHC IIA mRNA expression in the BF in response to ractopamine could result from a conservation mechanism wherein less MHC IIA isoform is degraded, leading to decreases in need for myofibrillar protein synthesis; however, in vitro protein degradation rates in the BF were numerically increased and changes in calpastatin mRNA was not affected in BF. Ractopamine did not affect calpastatin mRNA expression in either BF or LM, although it did numerically increase calpastatin mRNA expression in LM ( $P = 0.19$ ). Calpastatin regulates the activity of m-calpain and  $\mu$ -calpain which are responsible for initiating myofibrillar protein disassembly and, therefore, changes in mRNA expression of calpastatin could reflect changes in protein degradation. Parr et al. (1992) observed increases in activity of m-calpain and calpastatin by 27 and 76% in LM of steers fed 1.5 ppm cimaterol for 16 wk. In addition, mRNA levels of m-calpain and calpastatin were increased by 30 and 96% due to cimaterol treatment. The authors were the first to report simultaneous up-regulation of m-calpain and calpastatin due to  $\beta$ -agonist feeding and suggest that this may be the physiological response observed when  $\beta$ -agonists are used. Koochmaraie et al. (1991) demonstrated a 63% increase in calpastatin activity whereas activities of  $\mu$ -calpain and m-calpain were not affected in the BF of lambs fed 4 ppm L<sub>644,969</sub> for 6 wk. We did not observe significant changes in expression of calpastatin mRNA, and the numeric response in LM

represented only a 19% increase. Benson et al. (1991) observed a reduction in 3-methylhistidine concentration in the medium at 0 and 2 h of incubation of soleus muscle removed from fasted rats treated with 2 ppm clenbuterol in the water for 2 d compared to control fasted rats, demonstrating a reduction in degradation of myofibrillar protein.

$\beta_1$ - and  $\beta_2$ -receptor mRNA expressions in the BF were not affected by treatment. Ractopamine tended to decrease  $\beta_1$ -receptor mRNA abundance in LM in steers on d 14 and in heifers on d 28 (gender  $\times$  ractopamine  $\times$  day interaction;  $P = 0.11$ ). Ractopamine decreased  $\beta_2$ -receptor mRNA expression in LM of steers on d 14, but increased it in steers on d 28 (gender  $\times$  ractopamine  $\times$  day interaction;  $P = 0.03$ ). Sissom et al. (2007) reported that, in the semimembranosus of implanted heifers, ractopamine did not affect  $\beta_1$ -receptor mRNA abundance and tended to increase  $\beta_2$ -receptor mRNA expression. Implanted yearling steers had increased  $\beta_2$ -receptor mRNA expression in semimembranosus when fed ractopamine for 28 d (Winterholler et al., 2007). Growing Holstein steers fed ractopamine for 28 d demonstrated decreases in  $\beta_1$ - and  $\beta_2$ -receptor mRNA expression in LM (Chapter 3 in this thesis). In BF, ractopamine numerically decreased mRNA expression of  $\beta_3$ -receptor in steers, whereas expression in heifers were not affected by treatment (gender  $\times$  ractopamine,  $P = 0.19$ ). Ractopamine did not affect  $\beta_3$ -receptor mRNA expression in growing Holstein steers (Chapter 3 of this thesis). Chronic exposure to  $\beta$ -agonists is suggested to lead to desensitization of the  $\beta$ -receptors (Hausdorff et al., 1990) resulting in loss of the  $\beta$ -receptors. We observed only numeric decreases in mRNA for  $\beta$ -receptors, although these numeric decreases were present for all 3 receptors in both muscles.

Data for RNA, DNA, and protein concentrations and protein:DNA, protein:RNA, and RNA:DNA ratios are presented in Table 4.7. No effect of ractopamine on RNA, DNA, and protein concentration was observed. Additionally, protein:DNA, protein:RNA, and RNA:DNA ratios were not affected by ractopamine. O'Connor et al. (1991) demonstrated 42 and 25% reductions in DNA concentrations, 25 and 12% increases in RNA concentrations, and 1 and 13% increases in protein concentrations in the ST of ram lambs fed 10 ppm cimaterol for 3 and 6 wk. Additionally, in that study, protein:DNA increased by 76 and 51%, protein:RNA decreased by 21 and 2%, and RNA:DNA was increased by 109 and 52%. Beermann et al. (1987) demonstrated a 22% decrease in DNA concentration in semitendinosus in lambs fed 10 ppm of cimaterol for 7 wk, and RNA concentrations in semitendinosus were increased by 9% and protein concentrations

were increased by 8.1% when lambs were fed cimaterol for 12 wk. Protein:DNA was increased by 22 and 8%, protein:RNA was decreased by 5 and 1%, and RNA:DNA was increased by 29 and 10% after 7 and 12 wk of cimaterol feeding. In our study, differences among treatments were not observed, which may reflect no true difference or an inability to detect modest differences that might have been present.

### ***Conclusions***

Our results suggest that ractopamine fed to steers and heifers implanted with TBA/E<sub>2</sub> impacts in vitro protein degradation rates differently between the LM and BF. Feeding ractopamine seems to change the muscle fiber composition in BF as shown by decreases in MHC IIA expression. The role of IGF-I, if any, in mediating the action of ractopamine warrants further research. Although there were a few criteria for which ractopamine led to differences in response between steers and heifers, there were no striking disparities to suggest that the effectiveness of ractopamine would markedly differ between genders.

## References

- Awede, B. L., J. P. Thissen, and J. LeBacq. 2002. Role of IGF-I and IGF-BPs in the changes of mass and phenotype induced in rat soleus muscle by clenbuterol. *Am. J. Physiol. Endocrinol. Metab.* 282:E31-E37.
- Beermann, D. H., W. R. Butler, D. E. Hogue, V. K. Fishell, R. H. Dalrymple, C. A. Ricks, and C. G. Scanes. 1987. Cimaterol-induced muscle hypertrophy and altered endocrine status in lambs. *J. Anim. Sci.* 65:1514-1524.
- Benson, D. W., T. Foley-Nelson, W. T. Chance, F. S. Zhang, J. H. James, and J. E. Fischer. 1991. Decreased myofibrillar protein breakdown following treatment with clenbuterol. *J. Surg. Res.* 50:1-5.
- Bergen, W. G., S. E. Johnson, D. M. Skjaerlund, A. S. Babiker, N. K. Ames, R. A. Merkel, and D. B. Anderson. 1989. Muscle protein metabolism in finishing pigs fed ractopamine. *J. Anim. Sci.* 67:2255-2262.
- Blin, N., and D. W. Stafford. 1976. A general method for isolation of high molecular weight DNA from eukaryotes. *Nucleic Acid Res.* 3:2303-2308.
- Depreux, F. F. S., A. L. Grant, D. B. Anderson, and D. E. Gerrard. 2002. Paylean alters myosin heavy chain isoform content in pig muscle. *J. Anim. Sci.* 80:1888-1894.
- Duan, C. 2002. Specifying the cellular responses to IGF signals: roles of IGF-binding proteins. *J. Endocrinol.* 175:41-54.
- Dunn, J. D., B. J. Johnson, J. P. Kayser, A. T. Waylan, E. K. Sissom, and J. S. Drouillard. 2003. Effects of flax supplementation and a combined trenbolone acetate and estradiol implant on circulating insulin-like growth factor-I and muscle insulin-like growth factor-I messenger RNA levels in beef cattle. *J. Anim. Sci.* 81:3028-3034.
- Ellenberger, M. A., D. E. Johnson, G. E. Carstens, K. L. Hossner, M. D. Holland, T. M. Nett, and C. F. Nockels. 1989. Endocrine and metabolic changes during altered growth rates in beef cattle. *J. Anim. Sci.* 67:1446-1454.
- Greenwood, R. H., E. C. Titgemeyer, G. L. Stokka, J. S. Drouillard, and C. A. Loest. 2001. Effects of L-carnitine on nitrogen retention and blood metabolites of growing steers and performance of finishing steers. *J. Anim. Sci.* 79:254-260.



- Greig, P. D., S. J. Rozovski, D. H. Elwyn, and J. M. Kinney. 1986. Protein synthesis and degradation in biopsies of rat skeletal muscle. *J. Surg. Res.* 40:248-260.
- Gochman, N., and J. M. Schmitz. 1972. Application of a new peroxide indicator reaction to the specific, automated determination of glucose with glucose oxidase. *Clin. Chem.* 18:943-950.
- Gruber, S. L., J. D. Tatum, T. E. Engle, M. A. Mitchell, S. B. Laudert, A. L. Schroeder, and W. J. Platter. 2007. Effects of ractopamine supplementation on growth performance and carcass characteristics of feedlot steers differing in biological type. *J. Anim. Sci.* 85:1809-1815.
- Hausdorff, W. P., M. G. Caron, and R. J. Lefkowitz. 1990. Turning off the signal: desensitization of  $\beta$ -adrenergic receptor function. *FASEB J.* 4:2881-2889.
- Kamanga-Sollo, E., M. S. Pampusch, M. E. White, M. R. Hathaway, W. R. Dayton. 2005. Insulin-like growth factor binding protein (IGFBP)-3 and IGFBP-5 mediate TGF- $\beta$ - and myostatin-induced suppression of proliferation in porcine embryonic myogenic cell cultures. *Exp. Cell Res.* 311:167-176.
- Koohmaraie, M., S. D. Shackelford, N. E. Muggli-Cockett, and R. T. Stone. 1991. Effect of the  $\beta$ -adrenergic agonist L<sub>644,969</sub> on muscle growth, endogenous proteinase activities, and postmortem proteolysis in wether lambs. *J. Anim. Sci.* 69:4823-4835.
- Labarca, C., and K. Paigen. 1980. A simple, rapid, and sensitive DNA assay procedure. *Anal. Biochem.* 102:344-352.
- Laudert, S. B., G. J. Vogel, A. L. Schroeder, and W. J. Platter. 2007. Effect of ractopamine HCl on growth and carcass traits of finishing heifers fed to slaughter. *J. Anim. Sci.* 85(Suppl. 1):663. (Abstr.)
- Laudert, S. B., G. J. Vogel, A. L. Schroeder, W. J. Platter, and M. T. Van Koeving. 2004. The effect of Optaflexx on growth performance and carcass traits of steers. Optaflexx™ Exchange No. 4. Elanco Animal Health, Greenfield, IN.
- Lobley, G. E., V. Milne, J. M. Lovie, P. J. Reeds, and K. Pennie. 1980. Whole body and tissue protein synthesis in cattle. *Br. J. Nutr.* 43:491-502.
- Marsh, W. H., B. Fingerhut, and H. Miller. 1965. Automated and manual direct methods for determination of blood urea. *Clin. Chem.* 11:624-627.

- Mersmann, H. J. 1998. Overview of the effects of  $\beta$ -adrenergic receptors on animal growth including mechanism of action. *J. Anim. Sci.* 76:160-172.
- O'Connor, R. M., W. R. Butler, D. E. Hogue, and D. H. Beermann. 1991. Temporal pattern of skeletal muscle changes in lambs fed cimaterol. *Domest. Anim. Endocrinol.* 8:549-554.
- Pampusch, M. S., B. J. Johnson, M. E. White, M. R. Hathaway, J. D. Dunn, A. T. Waylan, and W. R. Dayton. 2003. Time course of changes in growth factor mRNA levels in muscle of steroid-implanted and nonimplanted steers. *J. Anim. Sci.* 81:2733-2740.
- Parr, T., R. G. Bardsley, R. S. Gilmour, and P. J. Buttery. 1992. Changes in calpain and calpastatin mRNA induced by  $\beta$ -adrenergic stimulation of bovine skeletal muscle. *Eur. J. Biochem.* 208:333-339.
- Plouzek, C. A., and A. Trenkle. 1991. Insulin-like growth factor-I concentrations in plasma of intact and castrated male and female cattle at four ages. *Domest. Anim. Endocrinol.* 8:73-79.
- Quinn, M. J., J. S. Drouillard, C. D. Reinhardt, A. S. Webb, J. M. Pozuelo, M. L. May, C. E. Walker, and S. J. Winterholler. 2006. The effects of dose and duration of ractopamine-HCl administration on finishing performance and carcass traits of non-implanted beef heifers. *J. Anim. Sci.* 84(Suppl. 1):120. (Abstr.)
- Schroeder, A., D. Hancock, D. Mowrey, S. Laudert, G. Vogel, and D. Polser. 2005a. Dose titration of Optaflexx<sup>®</sup> (ractopamine-HCl) evaluating the effects on growth performance in feedlot steers. *J. Anim. Sci.* 83(Suppl. 1):163. (Abstr.)
- Schroeder, A., D. Hancock, D. Mowrey, S. Laudert, G. Vogel, and D. Polser. 2005b. Dose titration of Optaflexx<sup>®</sup> (ractopamine-HCl) evaluating the effects on growth performance in feedlot heifers. *J. Anim. Sci.* 83(Suppl. 1):170. (Abstr.)
- Sissom, E. K., C. D. Reinhardt, J. P. Hutcheson, W. T. Nichols, D. A. Yates, R. S. Swingle, and B. J. Johnson. 2007. Response to ractopamine-HCl in heifers is altered by implant strategy across days on feed. *J. Anim. Sci.* 85:2125-2132.
- Vestergaard, M., P. Henckel, N. Oksbjerg, and K. Sejrsen. 1994. The effect of cimaterol on muscle characteristics, capillary supply, and metabolic potentials of longissimus and semitendinosus muscles of young Friesian bulls. *J. Anim. Sci.* 72:2298-2306.
- Waalkes, T. P., and S. A. Udenfriend. 1957. A fluorometric method for the estimation of tyrosine in plasma and tissues. *J. Lab. Clin. Med.* 50:733-736.

- Wheeler, T. L., and M. Koohmaraie. 1992. Effects of  $\beta$ -adrenergic agonist L<sub>644,969</sub> on muscle protein turnover, endogenous proteinase activities, and meat tenderness in steers. *J. Anim. Sci.* 70:3035-3043.
- Winterholler, S. J., G. L. Parsons, C. D. Reinhardt, J. P. Hutcheson, W. T. Nichols, D. A. Yates, R. S. Swingle, and B. J. Johnson. 2007. Response to ractopamine-hydrogen chloride is similar in yearling steers across days on feed. *J. Anim. Sci.* 85:413-419.
- Yang, F., B. J. Johnson, M. E. White, M. R. Hathaway, and W. R. Dayton. 1999. Effect of insulin-like growth factor (IGF)-I and Des (1-3) IGF-I on the level of IGF binding protein-3 and IGF binding protein-3 mRNA in cultured porcine embryonic muscle cells. *J. Cell. Physiol.* 178:227-234.
- Yimlamai, T., S. L. Dodd, S. E. Borst, and S. Park. 2005. Clenbuterol induces muscle-specific attenuation of atrophy through effects on the ubiquitin-proteasome pathway. *J. Appl. Physiol.* 99:71-80.

**Table 4.1** Composition of the diet fed to finishing steers and heifers

Item	% of DM
Steam-flaked corn	77.5
Alfalfa hay	10.0
Steep liquor	6.0
Solvent soybean meal	4.0
Limestone	1.56
Urea	0.50
Salt	0.30
Vitamin/mineral/drug premix <sup>1</sup>	0.14

<sup>1</sup> To provide per kilogram of diet DM: 60 mg of Mn, 60 mg of Zn, 10 mg of Cu, 0.6 mg of I, 0.25 mg of Se, 0.1 mg of Co, 2,650 IU of vitamin A, 30 mg of monensin (Rumensin 80, Elanco Animal Health, Greenfield, IN), and 9 mg of tylosin (Tylan 40, Elanco Animal Health).

**Table 4.2** Sequence of bovine specific PCR primers and TaqMan Probes to be used for determination of expression of mRNA of IGF-I, IGFBP-3 and -5,  $\beta_1$ -,  $\beta_2$ -, and  $\beta_3$ -adrenergic receptors, calpastatin, and myosin heavy chain IIA

Primer and Probes	Sequence (5' to 3')
IGF-I (Accession # X15726)	
Forward	TGTGATTTCTTGAAGCAGGTGAA
Reverse	AGCACAGGGCCAGATAGAAGAG
TaqMan probe	6FAM-GCCCATCACATCCTCCTCGCA-TAMRA
IGFBP-3 (Accession # M76478)	
Forward	CATTCCCAACTGCGACAAGA
Reverse	GCAGAAACCCCGCTTCT
TaqMan probe	6FAM-AGAAAAAGCAGTGCCGCCCTTCCAA-TAMRA
IGFBP-5 (Accession # M62782)	
Forward	TCTTCCGGCCCAAACACA
Reverse	TCTTTCTGCGGTCCTTCTTAC
TaqMan probe	6FAM-CGCATCTCCGAGCTGAAGGCTGAA-TAMRA
$\beta_1$ -adrenergic receptor (Accession # AF188187)	
Forward	GTGGGACCGCTGGGAGTAT
Reverse	TGACACACAGGGTCTCAATGC
TaqMan probe	6FAM-CTCCTTCTTCTGCGAGCTCTGGACCTC-TAMRA
$\beta_2$ -adrenergic receptor (Accession # NM_174231)	
Forward	CAGCTCCAGAAGATCGACAAATC
Reverse	CTGCTCCACTTGACTGACGTTT
TaqMan probe	6FAM-AGGGCCGCTTCCATGCCC-TAMRA
$\beta_3$ -adrenergic receptor (Accession # X85961)	
Forward	AGGCAACCTGCTGGTAATCG
Reverse	GTCACGAACACGTTGGTCATG
TaqMan probe	6FAM-CCCGGACGCCGAGACTCCAG-TAMRA
Calpastatin (Accession # X67333)	
Forward	CCCTGGATCAACTTTCTGACAGT
Reverse	TGACTTTATCCTCTACAGGTTTATTCTCA
TaqMan probe	6FAM-TCGGGCAAAGACAGCCTGATCCA-TAMRA
Myosin heavy chain IIA (Accession # AB059398)	
Forward	CCCCGCCCCACATCTT
Reverse	TCTCCGGTGATCAGGATTGAC
TaqMan probe	6FAM-TCTCTGACAACGCCTATCAGTTCAT-TAMRA

**Table 4.3** Effect of ractopamine on growth characteristics of finishing steers and heifers

Item	No ractopamine		Ractopamine, 200 mg/d		SEM	<i>P</i> -value <sup>1</sup>		
	Heifers	Steers	Heifers	Steers		G	R	G × R
Initial BW, kg	476	509	471	516	6.9	<0.001	0.93	0.22
Final BW, kg	516	548	503	561	10.2	<0.001	0.99	0.07
DMI, kg/d	8.42 <sup>y</sup>	8.58 <sup>y</sup>	7.07 <sup>x</sup>	8.33 <sup>y</sup>	0.37	0.01	0.007	0.05
ADG, kg	1.42	1.41	1.17	1.64	0.18	0.17	0.94	0.15
G:F	0.169	0.159	0.164	0.193	0.019	0.59	0.42	0.28
G:F above maintenance <sup>2</sup>	0.302	0.287	0.337	0.372	0.033	0.76	0.07	0.44

<sup>1</sup> G = gender, R = ractopamine.

<sup>2</sup> Calculated with feed (2.14 Mcal NE<sub>m</sub>/kg) necessary to meet NE<sub>m</sub> requirements subtracted from DMI; NE<sub>m</sub> requirements = 0.077

$$\text{Mcal} \times (\text{BW} \times 0.96)^{0.75}.$$

<sup>x,y</sup> Means in rows not bearing a common superscript differ, *P* < 0.05.

**Table 4.4** The effect of gender on blood metabolites, protein turnover, and gene expression

Item	Heifers	Steers	SEM	<i>P</i> -value
Serum	--- ng/mL ---			
IGF-I	488	695	29	<0.001
Insulin	1.02	1.32	0.21	0.32
Plasma	--- mmol/L ---			
Urea-N	5.26	4.59	0.16	0.003
Glucose	5.5	6.5	0.39	0.08
Protein turnover				
Protein synthesis	--- µg Tyr/(g wet tissue × h) ---			
Biceps femoris	0.313	0.309	0.011	0.76
LM	0.291	0.304	0.0093	0.10
Protein degradation	--- µg Tyr/(g protein × h) ---			
Biceps femoris	92	86	5.9	0.42
LM	68	66	3.9	0.64
Gene expression				
Biceps femoris	--- Arbitrary units, 10 <sup>-6</sup> ---			
IGF-I	4.0	5.3	0.44	0.05
IGFBP-3	172	193	22	0.32
IGFBP-5	702	846	89	0.26
Calpastatin	201	209	16	0.59
Myosin heavy chain IIA	1355	1455	149	0.56
β <sub>1</sub> -adrenergic receptor	0.71	0.98	0.21	0.37
β <sub>2</sub> -adrenergic receptor	113	128	16	0.38
β <sub>3</sub> -adrenergic receptor	3.4	5.5	0.94	0.08
LM				
IGF-I	4.2	3.8	0.40	0.33
IGFBP-3	169	217	25	0.10
IGFBP-5	640	801	83	0.17
Calpastatin	179	207	16	0.22
Myosin heavy chain IIA	1694	1594	128	0.59
β <sub>1</sub> -adrenergic receptor	0.56	0.54	0.15	0.92
β <sub>2</sub> -adrenergic receptor	86	108	15	0.11
β <sub>3</sub> -adrenergic receptor	3.1	3.4	1.0	0.79

**Table 4.5** The effect of ractopamine and gender on blood metabolites, protein turnover, and gene expression in finishing steers and heifers

Item	Day 14				Day 28				SEM	<i>P</i> -values <sup>1</sup>				
	No ractopamine		200 mg/d ractopamine		No ractopamine		200 mg/d ractopamine			R	R × D	G × D	G × R	G × R × D
	Heifers	Steers	Heifers	Steers	Heifers	Steers	Heifers	Steers						
Serum	--- ng/mL ---													
IGF-I	669 <sup>z</sup>	691 <sup>z</sup>	607 <sup>y</sup>	618 <sup>y,z</sup>	578 <sup>x,y</sup>	534 <sup>x</sup>	505 <sup>x</sup>	680 <sup>z</sup>	44	0.51	0.04	0.33	0.04	0.03
Insulin	1.39	1.39	1.10	1.50	1.03	1.16	1.20	1.17	0.32	0.99	0.39	0.49	0.80	0.20
Plasma	--- mmol/L ---													
Urea-N	4.45	4.60	4.02	4.18	5.81	5.28	5.27	5.26	0.26	0.06	0.55	0.08	0.47	0.30
Glucose	5.0	6.6	5.1	5.5	5.3	7.6	5.6	6.0	1.0	0.34	0.93	0.77	0.20	0.75
Protein turnover														
Protein synthesis	--- μg Tyr/(g wet tissue × h) ---													
Biceps femoris	0.319	0.333	0.329	0.323	0.276	0.270	0.291	0.287	0.017	0.53	0.41	0.64	0.71	0.56
LM	0.312	0.318	0.304	0.328	0.257	0.286	0.263	0.259	0.014	0.58	0.38	0.88	0.69	0.07
Protein degradation	--- μg Tyr/(g protein × h) ---													
Biceps femoris	87	81	110	85	92	74	107	79	12	0.19	0.75	0.56	0.42	0.66
LM	66	74	62	59	73	68	56	64	7	0.03	0.85	0.88	0.96	0.13
Gene expression	--- Arbitrary units, 10 <sup>-6</sup> ---													
Biceps femoris														
IGF-I	3.1	5.5	5.3	5.7	3.0	4.2	4.3	4.8	1.0	0.21	0.76	0.55	0.40	0.52
IGFBP-3	116	172	135	145	214	243	192	188	46	0.42	0.44	0.65	0.43	0.90
IGFBP-5	530	877	728	565	742	796	658	771	142	0.61	0.99	0.96	0.29	0.90
Calpastatin	107	183	141	164	237	205	240	214	38	0.73	0.97	0.13	0.54	0.57
Myosin heavy chain IIA	1314	1489	948	837	1279	1336	906	1347	254	0.04	0.31	0.50	0.88	0.30
β <sub>1</sub> -adrenergic receptor	0.24	1.85	0.18	0.57	0.52	0.47	0.64	0.31	0.7	0.38	0.39	0.12	0.33	0.54
β <sub>2</sub> -adrenergic receptor	69	15	62	114	118	117	123	112	35	0.62	0.58	0.10	0.64	0.78
β <sub>3</sub> -adrenergic receptor	1.4	8.4	1.2	3.3	2.3	5.8	2.5	2.4	2.9	0.22	0.77	0.46	0.19	0.86
LM														
IGF-I	3.7	3.6	6.1	3.8	2.9	4.2	3.9	3.5	1.0	0.22	0.31	0.16	0.11	0.83
IGFBP-3	100	267	193	258	214	221	191	197	58	0.80	0.29	0.08	0.48	0.42
IGFBP-5	440	1145	703	807	594	755	531	716	222	0.75	0.96	0.36	0.30	0.22
Calpastatin	115	228	191	213	195	192	223	256	43	0.19	0.78	0.33	0.63	0.24
Myosin heavy chain IIA	1010	1434	1262	1105	1624	1961	1978	1687	353	0.99	0.81	0.73	0.18	0.94
β <sub>1</sub> -adrenergic receptor	0.22	0.92	0.32	0.13	0.80	0.53	0.46	0.60	0.4	0.35	0.59	0.42	0.64	0.11
β <sub>2</sub> -adrenergic receptor	58 <sup>x</sup>	194 <sup>y</sup>	68 <sup>x</sup>	65 <sup>x</sup>	103 <sup>x</sup>	106 <sup>x</sup>	104 <sup>x</sup>	138 <sup>x</sup>	30	0.28	0.05	0.23	0.17	0.03
β <sub>3</sub> -adrenergic receptor	2.1	7.3	2.5	1.1	3.5	4.7	3.1	2.9	2.1	0.26	0.26	0.36	0.25	0.10

<sup>1</sup> R = ractopamine, D = day, G = gender. <sup>x,y,z</sup> Means in rows not bearing a common superscript differ, *P* < 0.05.



**Table 4.6** The effect of ractopamine on blood metabolites, protein turnover, and gene expression

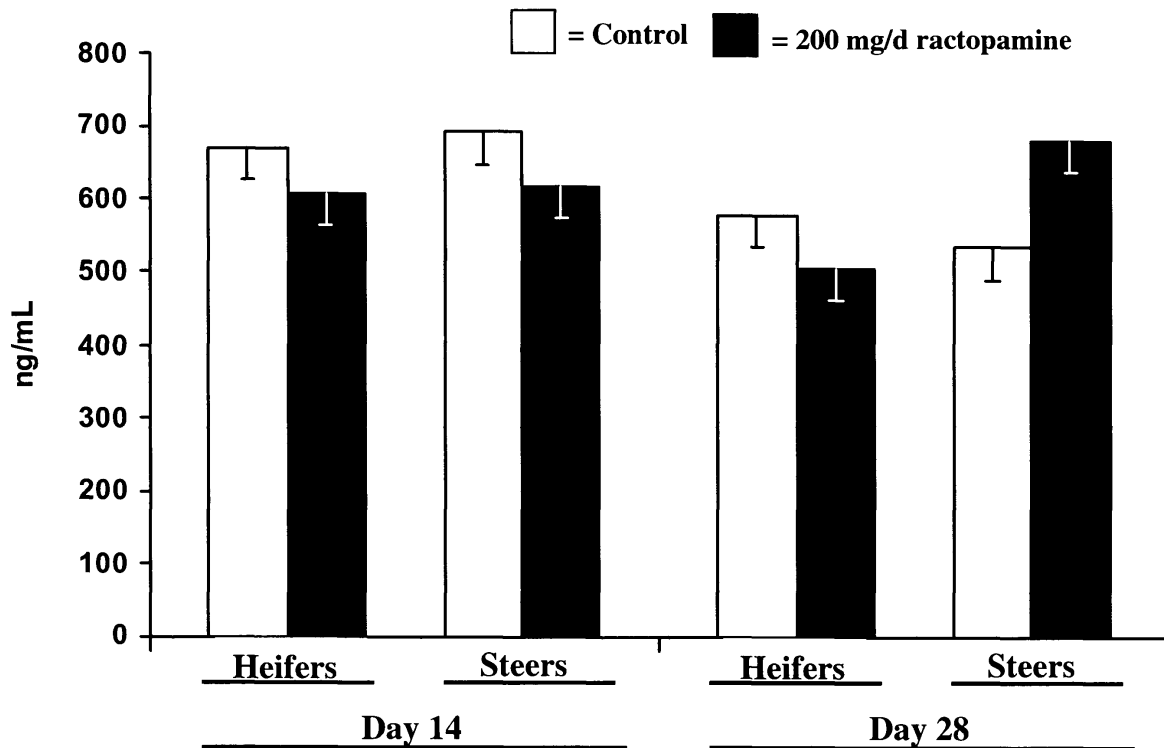
Item	No		SEM	P-value
	ractopamine	200 mg/d ractopamine		
Serum	--- ng/mL ---			
IGF-I	618	602	20	0.51
Insulin	1.24	1.24	0.22	0.99
Plasma	--- mmol/L ---			
Urea-N	5.03	4.65	0.15	0.06
Glucose	6.1	5.5	0.44	0.34
Protein turnover				
Protein synthesis	--- $\mu\text{g Tyr}/(\text{g wet tissue} \times \text{h})$ ---			
Biceps femoris	0.300	0.307	0.011	0.53
LM	0.293	0.288	0.011	0.58
Protein degradation	--- $\mu\text{g Tyr}/(\text{g protein} \times \text{h})$ ---			
Biceps femoris	84	95	7.1	0.19
LM	70	60	4.1	0.03
Gene expression				
Biceps femoris	--- Arbitrary units, $10^{-6}$ ---			
IGF-I	3.93	5.02	0.59	0.21
IGFBP-3	186	165	30	0.42
IGFBP-5	736	680	80	0.61
Calpastatin	183	190	19	0.73
Myosin heavy chain IIA	1354	1009	152	0.04
$\beta_1$ -adrenergic receptor	0.77	0.43	0.28	0.38
$\beta_2$ -adrenergic receptor	115	103	17	0.62
$\beta_3$ -adrenergic receptor	4.5	2.3	1.2	0.22
LM				
IGF-I	3.60	4.32	0.41	0.22
IGFBP-3	200	210	27	0.80
IGFBP-5	734	689	103	0.75
Calpastatin	183	221	20	0.19
Myosin heavy chain IIA	1507	1508	159	0.99
$\beta_1$ -adrenergic receptor	0.62	0.38	0.22	0.35
$\beta_2$ -adrenergic receptor	115	94	15	0.28
$\beta_3$ -adrenergic receptor	4.4	2.4	1.5	0.26

**Table 4.7** Effects of ractopamine and gender on RNA, DNA, and protein concentrations and protein:DNA, protein:RNA, and RNA:DNA ratios in the biceps femoris and LM on day 28

Item	No Ractopamine		200 mg/d ractopamine		SEM	<i>P</i> -value <sup>1</sup>		
	Heifers	Steers	Heifers	Steers		G	R	G × R
Biceps femoris								
RNA concentration, mg/g	0.199	0.206	0.200	0.209	0.014	0.62	0.90	0.94
DNA concentration, mg/g	0.220	0.207	0.181	0.210	0.023	0.71	0.40	0.32
Protein concentration, mg/g	103	102	103	101	4.2	0.65	0.91	0.85
Protein:DNA	512	563	573	556	72	0.80	0.69	0.62
Protein:RNA	535	531	529	513	46	0.82	0.77	0.88
RNA:DNA	1.02	1.15	1.10	1.12	0.16	0.60	0.86	0.67
LM								
RNA concentration, mg/g	0.193	0.193	0.186	0.203	0.013	0.42	0.84	0.41
DNA concentration, mg/g	0.161	0.171	0.161	0.166	0.025	0.74	0.90	0.92
Protein concentration, mg/g	100	107	97	101	3.2	0.11	0.11	0.62
Protein:DNA	686	860	698	634	128	0.65	0.38	0.33
Protein:RNA	529	598	525	508	50	0.55	0.29	0.33
RNA:DNA	1.33	1.44	1.37	1.30	0.23	0.91	0.72	0.60

<sup>1</sup>G = gender, R = ractopamine.

## Serum IGF-I



**Figure 4.1** Serum IGF-I concentrations in heifers and steers fed diets with 0 or 200 mg/d ractopamine.

The use of d 0 data in covariate analysis removed much of the gender effects. Gender  $\times$  Ractopamine  $\times$  Day interaction,  $P = 0.03$ .

## Appendix A -



1111 NORTH DUNLAP AVENUE  
SAVOY, IL 61874  
PHONE: (217) 356-9050  
FAX: (217) 398-4119  
E-MAIL: ASAS@ASSOCHQ.ORG  
WEB: WWW.ASAS.ORG

*EDITOR-IN-CHIEF*  
DR. LAWRENCE P. REYNOLDS  
NORTH DAKOTA STATE UNIVERSITY  
(701) 231-7646  
LARRY.REYNOLDS@NDSU.EDU

*MANAGING EDITOR*  
SUSAN POLLOCK  
EDITORIAL OFFICE  
OFFICE: 217.356.7641  
FAX: 217.378.4083  
SUSANP@ASSOCHQ.ORG

*JOURNALS ADMINISTRATIVE ASSISTANT*  
JEREMY HOLZNER  
EDITORIAL OFFICE  
217.356.2426 EXT. 38  
JEREMYH@ASSOCHQ.ORG

November 30, 2007

Dillon Walker  
Kansas State University  
119 Call Hall  
Manhattan, KS 66506

Dear Mr. Walker:

The *Journal of Animal Science* grants you nonexclusive permission to print the article listed below in your dissertation, provided that appropriate credit is given to the original publication in the journal.

D. K. Walker, E. C. Titgemeyer, J. S. Drouillard, E. R. Loe, B. E. Deppenbusch, and A. S. Webb; 2006; Effects of ractopamine and protein source on growth performance and carcass characteristics of feedlot heifers. *J. Anim. Sci.* 84:2795-2800.

Authors typically use a statement along the lines of "Used by permission of the *Journal of Animal Science*" and include identifying information, such as year, volume, and pages.

Best regards,

Susan Pollock  
Managing Editor

*LEADING SOURCE OF NEW KNOWLEDGE AND PERSPECTIVE IN ANIMAL SCIENCE*  
PUBLISHER OF THE *JOURNAL OF ANIMAL SCIENCE*

## Appendix B -

Dear Dillon Walker,

Thank you for your email request. Permission is granted for you to use the material below for your thesis/dissertation subject to the usual acknowledgements and on the understanding that you will reapply for permission if you wish to distribute or publish your thesis/dissertation commercially.

Best wishes,

*Lina Kopicaite*

**From:** Dillon Walker [mailto:dillonw@ksu.edu]

**Sent:** 30 November 2007 22:09

**To:** Journals Rights

**Subject:** Copyright permission

I would like permission to publishing the following article in my Ph.D. dissertation:

**Effects of steroidal implantation and ractopamine-HCl on nitrogen retention, blood metabolites and skeletal muscle gene expression in Holstein steers.** D. K. Walker, E. C. Titgemeyer, E. K. Sissom, K. R. Brown, J. J. Higgins, G. A. Andrews, and B. J. Johnson. J. Anim. Physiol. Anim. Nutr. Volume 91 pages 439-447, 2007.

My contact information is;

Dillon Walker

Kansas State University

119 Call Hall

Manhattan, KS 66506

email: [dillonw@ksu.edu](mailto:dillonw@ksu.edu)

Regards,

Dillon