CHANGES IN GROWTH PERFORMANCE AND CRITICAL COMPONENTS OF THE SOMATOTROPIC GROWTH AXIS IN GROWING PIGS AFTER INFECTION WITH SALMONELLA ENTERICA SEROVAR TYPHIMURIUM OR CHOLERAESUIS

by

BRIAN LEE DAVIS

B.S., Kansas State University, 2004

A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Animal Sciences and Industry
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2008

Approved by:

_____
Major Professor
J. Ernest Minton

ABSTRACT

Enteric disease and immune challenge are processes that have detrimental effects on growth performance of young swine. The current study tested the hypothesis salmonellae-induced enteric disease would perturb the endocrine growth axis in a serovar dependent fashion. Specifically, we evaluated effects of *Salmonella enterica* serovar Typhimurium (Typhimurium) and serovar Choleraesuis (Choleraesuis) on critical regulatory components of growth in young swine. Weaned pigs were housed 2 per pen with *ad libitum* access to feed and water in a 14 d experiment. Pigs were then repeatedly fed either 10^8 CFU Choleraesuis or 10^8 Typhimurium in dough balls, with control pigs receiving dough without bacteria. Bacteria were re-fed twice weekly. Rectal temperatures were monitored daily from d 0 to7 and ADFI was measured through d 14. Pigs were weighed and samples of serum were obtained for circulating IGF-I on days 0, 7, and 14. At the conclusion of the study, samples of semitendinosus muscle and liver were obtained and subsequently assayed for IGF-I, IGFBP-3, and IGFBP-5 mRNA. Rectal temperatures were elevated in pigs given Choleraesuis pigs from d 2 through d 7 ($P < 0.05$) when compared to control pigs and pigs fed Typhimurium. Pigs receiving Choleraesuis had substantially decreased feed intake on days 2, 3, 4, 7, 8, 9, and 10 ($P < 0.01$), with a trend for reduction on d 5 ($P = .08$), and they experienced an approximate 25% reduction in BW compared to control and Typhimurium pigs by the conclusion of the study. Pigs given Choleraesuis also experienced marked reductions in circulating IGF-I on d 7 ($P < 0.01$ vs. control and Typhimurium) with reductions of lesser magnitude on d 14 ($P = 0.07$ vs. control and $P < 0.05$ vs. Typhimurium). Treatment tended to affect liver IGFBP-3 mRNA ($P = 0.08$), where expression tended to be elevated in

Typhimurium and Choleraesuis pigs. In contrast, IGFBP-3 mRNA relative abundance was increased ($P < 0.03$) in pigs given Typhimurium versus control pigs. Muscle IGF-I mRNA was reduced in Choleraesuis pigs compared to control and Typhimurium ($P < 0.05$). Treatment tended to affect muscle IGFBP-3 mRNA ($P = 0.10$), where Choleraesuis had numerically less relative abundance than controls. Oral inoculation of growing pigs with Choleraesuis disrupted feed intake and BW gain, and this was accompanied by decreases in circulating IGF-I and reduced muscle expression of mRNA for IGF-I and IGFBP-3.

TABLE OF CONTENTS

LIST OF FIGURES

ACKNOWELDGEMENTS

Firstly, I would like to thank my major professor Dr. J. Ernest Minton. Without you, I would not have been able to pursue and further my education here at Kansas State University. Your support, guidance, and above all, your patience have allowed me to excel and broaden my horizons as well as given me that extra boost to pursue all of the goals I have set out for myself.

I would also like to thank Dr. Bradley J. Johnson and Dr. Steve S. Dritz who served as two integral members of my supervisory committee. Both of you exhibited professionalism and a commitment to me and my research, and served as valuable resources both professionally and outside of academia.

A vast amount of thanks goes out to my fellow graduate students Thomas Burkey, Kris Skjolaas, and Jennifer Fraser. Your awesome help and unequaled generosity to me when I needed it are what allowed me to keep my sanity. Without each of you, I don't think I would have made this project possible. Special thanks also goes out to Colleen Hill. Without her knowledge, help, and gracious attitude, the lab would have seemed an endless supply of materials and protocols and I would have been lost had it not been for her.

Finally, and with most absolute heartfelt appreciation I want to thank my parents William and Cheryl Davis. You both have pushed me and supported me in everything I have done with my education. I could not have made it this far without both of you. I love you both and would never have accomplished all my dreams without your love, support, and guidance. Thank you both dearly.

Chapter 1

A Review of Critical Components of the Somatotropic Growth Axis and Concepts of Disease-Associated Growth Retardation

ABSTRACT

Enteric disease and immune challenge are processes that are accepted as contributors to diminished growth in animals, including swine. Studies conducted with numerous animal models of infection suggest that disease challenge results in activation of the immune system and redirection of dietary nutrients from normal growth and development towards supportive functions used to enhance the immune response. Research in the field of salmonellosis in swine has resulted in further data to support this idea. In the United States, two serovars of *Salmonella enterica* namely *Salmonella enterica* serovar Typhimurium (Typhimurium) and serovar Choleraesuis (Choleraesuis) account for nearly all cases of salmonellosis in swine. Each of these serovars has differing biological effects on the pig. In swine, each serovar invokes specific responses, with Typhimurium producing mainly self-limiting enteritis and Choleraesuis resulting in more serious and occasionally fatal septicemia. In light of the more localized enteric nature of physiologic responses of swine to Typhimurium and the apparent tendency of Choleraesuis to result in symptoms more in line with systemic disease, it is reasonable to assume that these serovars may have divergent effects on fundamental components regulating growth in young swine. This report aims to review published information on mechanisms of Typhimurium- and Choleraesuis-induced reductions in growth of swine and, for additional context, to more broadly consider other established enteric models of disease in animals and key components of regulatory pathways of animal growth.
KEYWORDS: Enteric Disease, Growth, Swine, Salmonella, Salmonella Typhimurium, Salmonella Choleraesuis

INTRODUCTION

One of the hallmarks of the pathophysiology of enteric disease is reduced growth performance. This reduction in growth is associated with changes in both the endocrine and local somatotropic growth axis (Jenkins *et al.,* 2004). Components of this axis include both central neuroendocrine and local autocrine and paracrine factors. Among these factors are: growth hormone (GH), the growth hormone receptor (GHR), the growth hormone binding proteins (GHBPs), insulin-like growth factors I and II (IGF-I and IGF-II), as well as the IGF receptors and the six known IGF binding proteins (IGFBPs) (Frago, 2005). The neuroendocrine and the immune systems interact to coordinate physiological responses to infection and inflammation. These adaptive responses include the induction of inflammatory mediators and fever, reduced feed intake with diminished growth performance, and development of a regulated and specific immune response to the pathogen. It is thought that this bi-directional communication may coordinate appropriate physiological responses in such a way that nonessential functions (e.g. growth) can be suspended temporarily while the host mounts a response that will return it to homeostasis (Balaji *et al.,* 2000). The detrimental effect of disease and immunological stress on growth rate and efficiency of gain in food animals is of considerable economic importance (Spurlock *et al.,* 1997) as it has been shown that immunological stress can reduce growth rate and feed efficiency from 10 to 25% (Webel *et al.,* 1997; Fraser *et al*., 2007). The emerging view is that the reduction in feed intake and growth observed in disease or immunologically challenged pigs is the result of increased cytokine secretion (Webel *et al.,* 1997) as it is established that inflammatory cytokines are associated with

decreased voluntary food intake, increase resting energy expenditure and body temperature, and altered nutrient metabolism (Dritz *et al.,* 1996). Cytokines are small nonstructural proteins, or glycoproteins that serve as chemical messengers between cells and are mainly involved in regulation of the immune response. However, recent evidence has shown that they are involved in different physiological processes such as cell growth and differentiation, tissue repair and remodeling, and aging (Zoico *et al.,* 2002). Cytokines can also influence different functions of skeletal muscle cells and are part of the normal adaptive response of the tissue to physical stress. They play an important role not only in muscle homeostasis, but also in disease pathogenesis (Zoico *et al.,* 2002). Pathological conditions, such as inflammatory disease, can result in a decrease in lean body mass. Elevated levels of systemic cytokines, such as IL-1, IL-6, and TNF-α are associated with these inflammatory conditions (Johnson *et al.,* 2005) and have been shown to decrease circulating and tissue levels of important anabolic hormones (Frost *et al.,* 2002) as well as invoke other immune modulators such as glucocortocoids, prostaglandins, and catecholamines, all of which may affect cell metabolism and growth (Spurlock *et al.,* 1997). Experimental findings with purified lipopolysaccharide (LPS), *Salmonella enterica* serovar Typhimurium (Typhimurium) and serovar Choleraesuis (Choleraesuis) have supported the hypothesis of inflammatory processes and negative effects on growth. This report aims to review current literature on the effects of immune challenge in various models, including oral inoculation with Typhimurium and Choleraesuis; to review the processes that may be involved in the characteristic decrease in growth performance that follows immune challenge; and to review the processes involved in normal growth and development in various animal models.

REGULATION OF GROWTH

Growth Hormone. Physiological processes such as growth are carefully orchestrated and controlled by complex interactions among a multiplicity of hormones and growth factors (Boyd, 1989). These factors can modulate growth both in an endocrine and local manner. Growth hormone (GH) is produced primarily, or almost exclusively, by somatotrophs of the anterior pituitary, and its synthesis and secretion is under the control of the hypothalamus via two hypothalamic factors: the stimulating factor known as growth hormone releasing hormone (GHRH) and the inhibiting factor being somatostatin (Frago, 2005). It is now firmly established that GH plays a key role in the regulation of mammalian growth (Peng *et al.,* 1996) and is a major regulator of metabolism (Breier *et al.,* 1999).

IGF-I. The effects of GH are mediated through insulin-like growth factors namely insulin-like growth factor I (IGF-I) and insulin-like growth factor II (IGF-II) (Oksbjerg *et al.,* 2004) with both IGFs and their receptors being found in a variety of tissues and cell types, as well as in plasma and other biological fluids (Peng *et al.*, 1996; Silha, 2005). Initially, the dogmatic view was that GH secreted from the pituitary gland acted on the liver where it stimulated specific receptors and initiated the synthesis of a compound, later known as IGF-I from hepatic tissues. In many mammals, including swine, the majority of IGF-I found in circulation is thought to be produced by hepatic tissue (Breier *et al.,* 1999; Fan *et al.,* 1994; Florini *et al.,* 1996; Oksbjerg *et al.,* 2004; Peng *et al.,* 1996). However, the liver is not the only source. Further research has shown that other tissues, such as skeletal muscle, are intrinsically capable of producing their own IGF-I (Breier *et al.,* 1999; Fan *et al.,* 1994; Florini *et al.,* 1996; Oksbjerg *et al.,*

2004; Peng *et al.,* 1996) and therefore seem to utilize both circulating as well as locally synthesized IGF-I in an autocrine/paracrine fashion (Adamo, 2005). This concept is important, as it has been documented that approximately two thirds of growth to normal size that occurs postnatally is attributed to the autocrine/paracrine production and utilization of IGF-I (Novakofski, 1993). Evidence for this concept originates from research conducted in mouse models, in which genes for hepatic IGF-I production are eliminated. Thus, these mice experience drastic reductions in circulating IGF-I, but still grow to normal body size (Sjogren *et al.,* 1999; Yakar *et al.,* 1999) and provide further strength to support the importance of locally produced IGF-I in postnatal growth and development. Regardless of the source, it is accepted that IGF-I is a well documented and important regulator of growth and development, particularly of skeletal muscle (Fernandez, 2005; Florini *et al.*, 1996; Sheffield-Moore *et al.*, 2004). IGF-I also is a potent agent that affects the proliferation of a wide variety of cell types (Frago, 2005; Hathaway *et al.*, 2003). In addition, IGF-I is recognized to influence various aspects of carbohydrate, lipid and protein metabolism (Fan *et al.,* 1994), and is considered a dominant factor in the postnatal growth and hypertrophy of skeletal muscle cells. Through actions such as enhancing satellite cell proliferation in muscle cells, DNA content related to postnatal myofiber hypertrophy is increased (Oksbjerg *et al.,* 2004) with this amount of available DNA related to the amount of protein synthesis that occurs in muscle fibers postnatally (Novakofski, 1993). Indeed it has been shown that addition of IGF-I to myotube cultures of several cell lines and primary satellite cells increases the rate of protein synthesis and decreases the rate of protein degradation (Oksbjerg *et al.,* 2004) with the decreases in degradation thought to be related to the ability of IGF-I to

inhibit actions of the ubiquitin-proteasome system (Johnson *et al.,* 2005). Collectively, IGF-I has been found to contribute to the increases seen in muscle size as growth occurs, and has been shown to have effects on myoblast proliferation and stimulation of differentiation, as well as the stimulation of nutrient uptake and inhibition of proteolysis (Novakofski, 1993).

The mechanisms behind how IGF-I is regulated, both in circulation and local production are still under investigation, however it is accepted that IGF-I secretion from the liver, being the endocrine form found in plasma, is mostly dependent on the amount of GH secreted from the anterior pituitary (Adamo, 2005), with a negative feedback relationship occurring between IGF-I and GH, in which increasing amounts of IGF-I in circulation from the liver lead to decreasing amounts of GH release from the anterior pituitary (Scanes, 1995). It has also been found that the rate of GH secretion is related to nutrition, and that the amount of IGF-I produced by the liver is dependent on the number of receptors for GH found on hepatic tissue (Breier *et al.*, 1999; Scanes, 1995; Thissen, 2005). IGF-I production at tissues other than hepatic tissue, specifically skeletal muscle, is also related to GH secretion (Breier *et al.*, 1999; Florini *et al.*, 1996; Scanes, 1995; Sheffield-Moore *et al.*, 2004) but there has been some controversy as to the magnitude of the role of GH. For example, cultured myoblasts release IGFs intrinsically, but the relationship to GH treatment remains unclear, as there are conflicting studies examining the relationship between GH treatment and expression of IGFs. However, it remains a central principle that IGF expression in other tissues, including skeletal muscle, can occur both in the presence of GH and in its absence, although fundamental details of the regulation are yet to be clearly defined (Fernandez, 2005). Nevertheless, it is clear that a

relationship exists between GH and IGF-I. Overall the currently accepted view is that IGF-I, whether in circulation or intrinsically produced, is a potent mediator of GH and a necessary regulatory factor in the development of skeletal muscle and other tissues within the body.

IGFBPs. IGF's found in plasma and extracellular fluids exist predominantly in a complex with insulin-like growth factor binding proteins (IGFBPs) which control the bioavailability of the IGFs, and modulate their binding to target cell receptors. These IGFBP's are responsible for harboring IGF-I in circulation, thereby expanding its half-life and modulating its action on certain receptors for various tissues in the body (Florini *et al.*, 1996; Peng *et al.*, 1996; Silha, 2005). Outside of circulation, most target tissues also express IGFBPs as well as receptors for them, where they further regulate the local actions of the IGFs (Fernandez, 2005). In addition, all six IGFBPs found in circulation are also produced by individual tissues in a cell-type specific combination, with IGFBPs -2, -4, -5, and -6, found to be produced by skeletal muscle (Fernandez, 2005). Whereas this review will not cover the discovery and subsequently identified functions of each of the six known binding proteins in detail, focus will be on IGF binding protein 3 (IGFBP-3) and IGF binding protein 5 (IGFBP-5), because they are two binding proteins thought to play the largest role in the growth and development of porcine skeletal muscle; to mediate growth effects of IGF-I; and, therefore, to be subject to modulation of growth by enteric disease. Moreover, it has been well documented that these two binding proteins are responsible for sequestering more than 90% of IGF-I in circulation (Thissen, 2005) and that they form a ternary complex with an acid labile sub-unit (ALS) (Sandhu, 2005; Scanes, 1995; Silha, 2005; Thissen, 2005).

IGFBP-3. IGFBP-3 is the most abundant IGFBP in circulation (Silha, 2005) and is the major circulating carrier of IGF-I in postnatal life (Spagnoli, 2005). It is also a key component of the ternary complex made up of IGF-I, IGFBP-3, and ALS (Roberts, 2005; Silha, 2005). It is heavily expressed in hepatic tissues, but additionally is synthesized in other tissues including skin, skeletal muscle, reproductive organs, bone, and endothelium and has a central role in the regulation of IGF transport to tissues (Silha, 2005). IGFBP-3 sequesters between 75 to 90% of IGF-I in circulation (Sandhu, 2005), and like most other IGFBPs, the function of IGFBP-3 is to prolong the half life of IGF-I, act as a transport protein across the capillary barrier of tissues, and modulate presentation of IGF-I to its receptors on these tissues (Spagnoli, 2005). Interestingly though, IGFBP-3 also has a broad range of functions that are independent of its binding to IGF-I, including the control of cell proliferation, controlling glucose uptake, and even inducing or enhancing cell apoptosis (Spagnoli, 2005). Furthermore, a role for IGFBP-3 in various types of cancers, in to tumor suppression and in anti-apoptotic effects on various cell types has been proposed, although understanding of these functions continues to emerge (Lee *et al.*, 2002b).

In serum, IGFBP-3 concentrations are tightly regulated by a number of factors, but most importantly by the rate of its hepatic synthesis in the liver, a process which is substantially linked to the amount of IGF-I produced by the liver itself, and which in turn is related to the amount of GH released (Scanes, 1995; Silha, 2005). Other important factors for the regulation of serum IGFBP-3 include nutrient and dietary intake, as it has been shown that hepatic IGFBP-3 concentrations in serum become reduced during protein restriction or malnutrition (Silha, 2005) and that this effect may be mediated

primarily by increased concentrations of insulin, glucose, and amino acids in circulation (Thissen, 2005). Although chronic dietary restriction can result in decreasing levels of IGFBP-3 in circulation (Lee *et al.*, 2002a; Thissen, 2005), these changes are also often paralleled in hepatic tissue, where decreasing levels of IGFBP-3 mRNA are expressed (Thissen, 2005). Changes in the amount of IGFBP-3 in other tissues, such as skeletal muscle for example, have been more difficult to quantify as the cellular mechanisms behind IGFBP-3 regulation have not been fully elucidated and remain largely undetermined (Granata *et al.,* 2004; Takaoka *et al.,* 2004). Thus, IGFBP-3 is an important mediator of the biological actions of IGF-I, but has several other important functions independent of those mediated by binding to IGF-I, including participation and regulation of processes such as cell growth, differentiation, and proliferation.

IGFBP-5. Like IGFBP-3, IGFBP-5 is capable of forming a ternary complex with IGF-I and is in part responsible for binding and mediating its effects, with approximately 58% of it present in circulation in conjunction with IGF-I and ALS (Silha, 2005; Thissen, 2005). IGFBP-5 also contributes to homeostasis in bone, ovary, kidney and skeletal muscle (Silha, 2005). It is intrinsically produced and secreted by cultured muscle cells (Fernandez, 2005), the functions and regulatory pathways of which are currently under study. In addition, IGFBP-5 appears to be associated with the differentiation of cultured muscle cells, and it may play a role in myogenesis (Florini *et al.,* 1996). Much like all other known IGFBPs, it appears to be tightly regulated by nutrition (Thissen, 2005). IGFBP-5 is also protected via IGF-I that it is bound to, in addition to the stimulation of its transcription by IGF-I and GH (Silha, 2005). It has been suggested that the regulation of expression of IGFBP-5, at least in proliferating porcine satellite cells, was also affected

by transforming growth factor β1 (TGFβ1), as IGFBP-5 mRNA expression was increased by 50%, suggesting another possible pathway for the control of expression (Oksbjerg *et al.,* 2004). However, it is important to note that the precise functions and nutritionally-regulated mechanisms for IGFBP-5 expression are largely unknown (Thissen, 2005). Much like IGFBP-3 however, IGFBP-5 has also participates in various biological processes independent of binding or interacting with IGF-I. Specifically, IGFBP-5 plays a major role in bone growth (Franchimont *et al.*, 1997; Schneider *et al.*, 2002) and participates to varying degrees in mammary gland involution, both follicular growth and atresia, in kidney development and function, and similar to IGFBP-3, in various types of cancers (Schneider *et al.*, 2002).

CYTOKINE MEDIATED EFFECTS ON COMPONENTS OF THE SOMATOTROPIC GROWTH AXIS

Reductions in body weight gain in growing pigs in response to enteric challenge with Typhimurium or Choleraesuis appear to be largely related to disease-associated depression in feed intake. Of particular importance to research models is the view that cytokines produce periods of inappetence and lethargy are associated with decreased feed intake and overall poor nutrient utilization (Johnson, 1997; Kelley, 1993; Spurlock *et al.*, 1997). These reviews probed the hypothesis that certain inflammatory cytokines, generated in response to an enteric pathogen by the host immune system, including TNF-α, IL-1, and IL-6, may be part of the underlying cause and that interactions between the immune system and disease challenge may be responsible for these negative effects. Therefore, certain components of the somatotropic growth axis known to regulate normal growth and development, including GH, IGF-I, IGFBP-3, and IGFBP-5, have been

implicated in disease challenge models to further clarify the interaction between immune challenge and growth.

EFFECTS ON GH AND GHR. It is accepted that GH plays a key role in the regulation of mammalian growth (Peng *et al.,* 1996), therefore the study of the effects that a disease challenge presents on GH is relevant. Normal, pulsatile secretion of GH from somatotrophs in the anterior pituitary is an important regulator of normal growth and development (Frago, 2005). The relationship between immune challenge, depressed growth and GH is likely being multifaceted (Spurlock *et al.,* 1997).

Although results of disease models, such as injection of LPS, have documented variable effects on GH secretion in domestic farm animals (increased in sheep: Coleman *et al*., 1993; transiently increased in pigs: Wright *et al*., 2000; decreased in cattle: Elsasser *et al*., 1988, 1995), relatively few studies have evaluated secretion of GH in response to bona fide disease challenge. Weaned pigs challenged orally with 10^9 CFU of Typhimurium experienced intermittent elevations of GH in serum compared with their control group counterparts (Balaji *et al*., 2000). The transient elevations in GH in that study may have been associated with decreased nutrient intake and the accompanying decrease in circulating IGF-I.

Nutritional status plays a major role in determining circulating GH concentrations as well as the negative effects that inadequate nutrition can have on the GH receptor in hepatic tissue, which has been established as being a critical link in IGF-I synthesis and, consequently, growth (Breier *et al.,* 1999). The role of cytokines, as they have been shown to negatively affect feed intake (Dantzer, 1993; Johnson, 1997), may therefore indirectly alter GH. Decreased feed intake can alter not only the amount and pattern of

GH secretion, but it has also been shown that food deprivation can decrease the number of GH receptors (GHR) on hepatic tissue as well as downstream receptor signaling. Amounts of GHR mRNA in liver tissue of steers that had either been food deprived and allowed a diet of water only were compared to a group of steers allowed free access to water, grass, and grain supplementation (Wang *et al.,* 2003). Analysis of liver tissues showed decreased GHR mRNA in the hepatic tissues of the food deprived steers. Paralleling these results, pig hepatocyte cultures were placed media containing an array of amino acids and glucose, or simply in culture with amino acids only (Brameld *et al.,* 1999). Results showed that hepatocytes in culture free of glucose had substantially less GHR expression than those exposed to glucose, again supporting the theory that nutrition, possibly glucose in particular, plays an important role in GHR expression and function. Thus, nutrient intake in response to disease challenge may play a key role in mediating the effects of GH partially through receptor expression in tissues, especially the liver. It also appears that inflammatory cytokine activity during an immune challenge, such as in the case of LPS or Salmonella infection, can affect nutritional status by affecting feed intake as part of the immune response, as well as alter nutrient utilization by shifting nutrients towards supporting immune function and away from growth promotion.

Although it has been established that nutrition plays a key role in mediating the effects of GH as well as being important for normal receptor signaling function, ongoing research has begun to elucidate the direct effects that inflammatory cytokines may have on GH as well as its receptor. An emerging parallel dogma is that nutrition may not be the only way in which GHRs are regulated, and that known inflammatory cytokines may have direct effects on the secretion of GH and furthermore effect GHR's in hepatic tissue.

It has been suggested that GH secretion may be directly affected by IL-1β by affecting the pulsatile release of GH as well as negatively affecting GH-releasing hormone (GHRH) secretion while upregulating the release of somatostatin, a known inhibitor of GH release (Spurlock *et al.,* 1997). This view is significant as it has been established that LPS, as well as salmonellae infections, lead to expression of IL-1, and a link between IL-1 and GH could explain a basis for decreased growth in swine. Another known inflammatory cytokine IL-6, can affect GHR post-receptor signaling in rat hepatic tissue through inhibition of the JAK/STAT dependent signaling pathway at different levels (Wang *et al.,* 2002a). In addition, TNF-α has also been implicated in reduced expression of liver GHR mRNA (Wang *et al.,* 2002b).

EFFECTS ON IGF-I. It has been widely accepted that the regulation of IGF-I production both by hepatic as well as in non-hepatic tissues is largely related to GH secretion (Adamo, 2005) as well as nutrition (Adamo, 2005; Breier *et al.*, 1999), but the effect of cytokines induced by an immune challenge have also been implicated in playing a role as well, both on circulating as well as tissue levels of IGF-I (Frost *et al.,* 2002).

In circulation, IGF-I is an important product of the liver, however, recent evidence suggests that not all of this IGF-I in circulation is a result of hepatic production, and that local production of IGF-I by non-hepatic tissues plays an important role is well, and is likely to contribute substantially to the final biologically active pool of IGF-I (Frost *et al.,* 2004). It is thought that inflammatory mediators, such as TNF-α, IL-1, or IL-6 may somehow affect production or regulatory pathways involved in IGF-I mediating the effects of GH. In particular, TNF-α may be major player in down regulating the effects of IGF-I. Cultured human myoblasts treated with TNF-α experienced reduced

ability for myoblasts to conduct protein synthesis for at least 48 hrs, but with no affect on the ability of IGF-I to bind to these cells, suggesting that TNF-α may affect some aspect of the IGF-I signal transduction pathway (Frost *et al.,* 1997). Incubation of TNF-α with C2C12 myoblasts resulted in progressive declines in total IGF-I mRNA levels through 10 hrs, suggesting that TNF-α may also hamper essential pathways for local IGF-I production in muscle cells (Fernandez-Celemin *et al.,* 2002). More recently, it was shown that TNF-α induced a state of receptor resistance in C2C12 myoblasts by affecting the docking proteins IRS-1 and IRS-2 that are critical for IGF-I to bind to its receptor (Broussard *et al.,* 2003). In a model of Porcine Reproductive and Respiratory Virus (PRRSV) disease in young pigs, systemic elevations of IL-1β and IL-6 that were negatively correlated with parameters such as feed intake, body weight gain, and protein accretion in muscles such as the biceps femoris, providing further evidence for the relationship between cytokines and their effects on feed intake as well as hampering the process of normal skeletal muscle growth and development (Escobar *et al.*, 2004). These studies taken together suggest that receptor resistance, coupled with decreased protein synthesis, decreased feed intake, and inhibition of local IGF-I production may be an explanation for the lack of muscle growth seen in animals under disease challenge, and suggest a mechanism for the inability of IGF-I to modulate the actions of GH.

EFFECTS ON IGFBP-3. IGFBP-3 appears to be potently regulated by nutrition as well as by the amount of IGF-I produced by the liver and the amount of GH secreted, which may be affected by known inflammatory cytokines IL-6 and TNF-α. It is accepted that IGFBP-3 regulation is directly related to feed intake as a result of the interaction between cytokines, feed intake, and nutritional effects on the IGF/GH axis in the liver.

However, knowledge about the direct affects that inflammatory cytokines may play on this important mediator of IGF-I function is generally lacking.

Circulating IGFBP-3 concentrations are reduced under conditions of restricted energy or feed intake (Lee *et al.*, 2002a). Pigs infected with 10^{10} CFU Typhimurium showed reductions in IGFBP-3 serum at 24, 48, 96, and 168 hours after infection (Jenkins *et al.,* 2004). Since IGFBP-3 is established as a primary sequestering agent for IGF-I, reduced concentrations of IGFBP-3 may enhance the availability of the remaining IGF-I to tissues (Breier *et al.,* 1999). Although data concerning the effects that inflammatory cytokines on IGFBP-3 are generally lacking, a role for IL-6 on IGFBP-3 has been suggested. Mice overexpressing IL-6 (NSE/hIL-6) showed a marked decrease in the amount of IGFBP-3 in circulation compared to their wild-type counterparts. Transgenic mice also showed marked increases in the proteolytic cleavage of IGFBP-3 in circulation, further showing that IL-6 may affect not only IGFBP-3 production, but that it may also play an important role in the degradation of existing IGFBP-3 (DeBenedetti *et al.,* 2001). In that same study, injections of exogenous recombinant human IL-6 to CB6F1 mice (non-transgenic counterparts of the same strain as the NSE/hIL6 mice) resulted in significant decreases in IGFBP-3 in circulation 24 hours after treatment as well. In addition, human patients with systemic juvenile idiopathic arthritis followed the same trend when compared to age matched controls, with decreased IGFBP-3 in serum as well as increased proteolysis of IGFBP-3 in the presence of high levels of IL-6, a distinguishing characteristic of this disease

Another inflammatory cytokine, TNF-α, has also been implicated in its ability to inhibit patters of growth in human muscle cells. Treatment of human myoblast cell

cultures with TNF-α inhibited IGFBP-3 secretion in a dose dependent manner, thereby blocking the ability for IGFBP-3 to induce myoblast differentiation and having further ramifications on skeletal muscle development and growth (Foulstone *et al.,* 2003). Collectively, evidence for the negative effects cytokines may have on IGFBP-3 is mounting, and further research may help advance understanding of the interactions that occur.

EFFECTS ON IGFBP-5. The most conserved IGFBP across species, IGFBP-5, plays an important role in several biological processes including bone, ovary, mammary gland, and kidney physiology (Schneider *et al.,* 2002) and is an important component of a ternary complex with ALS and IGF-I (Silha, 2005; Thissen, 2005). It is also the predominant IGFBP synthesized by skeletal muscle (Lang *et al.,* 2006). However, data concerning the direct effects that inflammatory mediators may have on IGFBP-5 function or expression are lacking. It is known that inflammatory cytokines IL-1 and IL-6 increase IGFBP-5 mRNA in bone cells (Franchimont *et al.,* 1997; Sunic *et al.,* 1998) but the effects of catabolic insult on IGFBP-5 in other tissues, particularly on skeletal muscle, that can occur as a result of sepsis remains relatively undetermined. Intraperitoneal injection of LPS in rats reduced IGFBP-5 mRNA concentrations in gastrocnemius muscle by 40-45% between 4-12 hrs after LPS, and the relationship was found to be dose dependent at the 8 h timepoint (Lang *et al.,* 2006). Cecal ligation and puncture also resulted in decreasd IGFBP-5 mRNA in gastrocnemius muscle again and increased serum levels of TNF-α (Lang *et al.,* 2006). In that same study, it was also found that continuous infusion of TNF-α resulted in significant decreases in IGFBP-5 mRNA as well, but these results were counteracted when rats were pretreated with $TNF_{BP,}$ a protein responsible for

binding TNF-α. Thus, increased concentrations of TNF-α appear to be associated with decreased IGFBP-5 expression in skeletal muscle. These data suggest that inflammatory challenge in a septic models has negative effects on IGFBP-5 production, and can thereby hamper IGFBP-5, an important protein responsible for normal muscle growth and development.

CONCLUSION

Inflammatory cytokines produced by an organism's immune system in response to infectious challenge can have dramatic effects on the central nervous system, alter behavior, and alter dominant components and control points of the somatotropic growth axis. It is these effects on behavior, nutrient deposition, and interactions with known promoters of growth and development, both in circulation and at local levels, that appear to be a driving force behind the deleterious effects of disease pathogenesis on growth and development.

REFERENCES

Adamo ML (2005). Overview and Molecular Aspects of the Insulin-Like Growth Factor System. In: Houston MS, Holly JMP and Feldman EL (eds) IGF and nutrition in health and disease. Totowa, N.J: Humana Press, pp. 1-22.

Balaji R, Wright KJ, Hill CM, Dritz SS, Knoppel EL and Minton JE (2000). Acute phase responses of pigs challenged orally with Salmonella typhimurium. *J. Anim. Sci.* **78**: 1885-1891.

Boyd DR and Bauman DE (1989). Mechanisms of Action for Somatotropin in Growth. In: Campion DR, Hausman GJ and Martin RJ (eds) Animal Growth Regulation. New York: Plenum Press, pp. 257-293.

Brameld JM, Gilmour RS and Buttery PJ (1999). Glucose and amino acids interact with hormones to control expression of insulin-like growth factor-I and growth hormone receptor mRNA in cultured pig hepatocytes. *J .Nutr.* **129**: 1298-1306.

Breier BH (1999). Regulation of protein and energy metabolism by the somatotropic axis. *Domest. Anim. Endocrinol.* **17**: 209-218.

Broussard SR, McCusker RH, Novakofski JE, Strle K, Shen WH, Johnson RW, Freund GG, Dantzer R and Kelley KW (2003). Cytokine-hormone interactions: tumor necrosis factor alpha impairs biologic activity and downstream activation signals of the insulin-like growth factor I receptor in myoblasts. *Endocrinology* **144**: 2988-2996.

Dantzer R (1993). Cytokines and Sickness Behavior. In: Husband AJ (eds) Psychoimmunology CNS-immune interactions. Boca Raton: CRC Press, pp. 1-16.

DeBenedetti F., Meazza C, Oliveri M, Pignatti P, Vivarelli M, Alonzi T, Fattori E, Garrone S, Barreca A and Martini A (2001). Effect of IL-6 on IGF binding protein-3: a study in IL-6 transgenic mice and in patients with systemic juvenile idiopathic arthritis. *Endocrinology* **142**: 4818-4826.

Dritz SS, Owen KQ, Goodband RD, Nelssen JL, Tokach MD, Chengappa MM and Blecha F (1996). Influence of lipopolysaccharide-induced immune challenge and diet complexity on growth performance and acute-phase protein production in segregated early-weaned pigs. *J. Anim. Sci.* **74**: 1620-1628.

Escobar J, Van Alstine WG, Baker DH and Johnson RW (2004). Decreased protein accretion in pigs with viral and bacterial pneumonia is associated with increased myostatin expression in muscle. *J. Nutr.* **134**: 3047-3053.

Fan J, Molina PE, Gelato MC and Lang CH (1994). Differential tissue regulation of insulin-like growth factor-I content and binding proteins after endotoxin. *Endocrinology* **134**: 1685-1692.

Fernandez AM and LeRoith D (2005). Skeletal Muscle. In: Varela-Nieto I and Chowen JA (eds) The growth hormone/insulin-like growth factor axis during development. New York: Springer, pp. 117-147.

Fernandez-Celemin L, Pasko N, Blomart V and Thissen JP (2002). Inhibition of muscle insulin-like growth factor I expression by tumor necrosis factor-alpha. *Am. J. Physio.l Endocrinol.Metab.* **283**: E1279-E1290.

Florini JR, Ewton DZ and Coolican SA (1996). Growth hormone and the insulin-like growth factor system in myogenesis. *Endocr. Rev.* **17**: 481-517.

Foulstone EJ, Savage PB, Crown AL, Holly JM and Stewart CE (2003). Role of insulin-like growth factor binding protein-3 (IGFBP-3) in the differentiation of primary human adult skeletal myoblasts. *J. Cell Physiol.* **195**: 70-79.

Frago LM and Chowen JA (2005). Basic Physiology of the Growth Hormone/Insulin-Like Growth Factor Axis. In: Varela-Nieto I and Chowen JA (eds) The growth hormone/insulin-like growth factor axis during development. New York: Springer, pp. 1-25.

Franchimont N, Durant D and Canalis E (1997). Interleukin-6 and its soluble receptor regulate the expression of insulin-like growth factor binding protein-5 in osteoblast cultures. *Endocrinology* **138**: 3380-3386.

Fraser JN, Davis BL, Skjolaas KA, Burkey TE, Dritz SS, Johnson BJ and Minton JE (2007). Effects of feeding *Salmonella enterica* serovar Typhimurium or serovar Choleraesuis on growth performance and circulating insulin-like growth factor-I, tumor necrosis factor-α, and interleukin-1ß in weaned pigs. *J. Anim. Sci* **85**: 1161-1167.

Frost RA and Lang CH (2004). Alteration of somatotropic function by proinflammatory cytokines. *J. Anim. Sci.* **82 E-Suppl**: E100-E109.

Frost RA, Lang CH and Gelato MC (1997). Transient exposure of human myoblasts to tumor necrosis factor-alpha inhibits serum and insulin-like growth factor-I stimulated protein synthesis. *Endocrinology* **138**: 4153-4159.

Frost RA, Nystrom GJ and Lang CH (2002). Lipopolysaccharide regulates proinflammatory cytokine expression in mouse myoblasts and skeletal muscle. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **283**: R698-R709.

Granata R, Trovato L, Garbarino G, Taliano M, Ponti R, Sala G, Ghidoni R and Ghigo E (2004). Dual effects of IGFBP-3 on endothelial cell apoptosis and survival: involvement of the sphingolipid signaling pathways. *FASEB J.* **18**: 1456-1458.

Hathaway MR, Dayton WR, White ME and Pampusch MS (2003). Effects of antimicrobials and weaning on porcine serum insulin-like growth factor binding protein levels. *J. Anim. Sci.* **81**: 1456-1463.

Jenkins NL, Turner JL, Dritz SS, Durham SK and Minton JE (2004). Changes in circulating insulin-like growth factor-I, insulin-like growth factor binding proteins, and leptin in weaned pigs infected with *Salmonella enterica* serovar Typhimurium. *Domest. Anim. Endocrinol.* **26**: 49-60.

Johnson BJ, Dritz SS, Skjolaas-Wilson KA, Burkey TA and Minton JE (2005). Interactive responses in gut immunity, and systemic and local changes in the insulin-like growth factor system in nursery pigs in response to *Salmonella enterica* serovar Typhimurium. *J. Anim. Sci.* 83:E48-56.

Johnson RW (1997). Inhibition of growth by pro-inflammatory cytokines: an integrated view. *J. Anim. Sci.* **75**: 1244-1255.

Kelley KW, Kent S and Dantzer R (1993). Why Sick Anim.als Don't Grow: An Immunological Explanation. In: Hollis GR (eds) Growth of the pig. Wallingford: CAB International, pp. 119-132.

Lang CH, Krawiec BJ, Huber D, McCoy JM and Frost RA (2006). Sepsis and inflammatory insults downregulate IGFBP-5, but not IGFBP-4, in skeletal muscle via a TNF-dependent mechanism. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **290**: R963-R972.

Lee CY, Lee HP, Jeong JH, Baik KH, Jin SK, Lee JH and Sohnt SH (2002a). Effects of restricted feeding, low-energy diet, and implantation of trenbolone acetate plus estradiol on growth, carcass traits, and circulating concentrations of insulin-like growth factor (IGF)-I and IGF-binding protein-3 in finishing barrows. *J. Anim. Sci.* **80**: 84-93.

Lee KW and Cohen P (2002b). Nuclear effects: unexpected intracellular actions of insulin-like growth factor binding protein-3. *J. Endocrinol.* **175**: 33-40.

Novakofski J and McCusker RH (1993). Physiology and Principles of Muscle Growth. In: Hollis GR (eds) Growth of the pig. Wallingford: CAB International, pp. 33-48.

Oksbjerg N, Gondret F and Vestergaard M (2004). Basic principles of muscle development and growth in meat-producing mammals as affected by the insulin-like growth factor (IGF) system. *Domest. Anim. Endocrinol.* **27**: 219-240.

Peng M, Pelletier G, Palin MF, Veronneau S, LeBel D and Abribat T (1996). Ontogeny of IGFs and IGFBPs mRNA levels and tissue concentrations in liver, kidney and skeletal muscle of pig. *Growth Dev. Aging* **60**: 171-187.

Roberts CT Jr (2005). The Role of the Insulin-Like Growth Factor System in Pre-and Postnatal Growth, Development, and Tumorigenesis. In: Houston MS, Holly JMP

and Feldman EL (eds) IGF and nutrition in health and disease. Totowa, N.J: Humana Press, pp. 121-132.

Sandhu MS (2005). Insulin-Like Growth Factor-I and Risk of Type 2 Diabetes and Coronary Heart Disease: Molecular Epidemiology. In: Cianfarani S, Clemmons DR and Savage MO (eds) IGF-I and IGF binding proteins basic research and clinical management. Basel: Karger, pp. 44-54.

Scanes CG and Daughaday WH (1995). Growth Hormone Action: Growth. In: Growth Hormone. Boca Raton: CRC Press, pp. 351-369.

Schneider MR, Wolf E, Hoeflich A and Lahm H (2002). IGF-binding protein-5: flexible player in the IGF system and effector on its own. *J. Endocrinol.* **172**: 423-440.

Sheffield-Moore M and Urban RJ (2004). An overview of the endocrinology of skeletal muscle. *Trends Endocrinol. Metab* **15**: 110-115.

Silha JV and Murphy LJ (2005). Insulin-Like Growth Factor Binding Proteins in Development. In: Varela-Nieto I and Chowen JA (eds) The growth hormone/insulin-like growth factor axis during development. New York: Springer, pp. 55-89.

Sjogren K, Liu JL, Blad K, Skrtic S, Vidal O, Wallenius V, LeRoith D, Tornell J, Isaksson OG, Jansson JO and Ohlsson C (1999). Liver-derived insulin-like growth factor I (IGF-I) is the principal source of IGF-I in blood but is not required for postnatal body growth in mice. *Proc. Natl. Acad. Sci. U.S.A* **96**: 7088-7092.

Spagnoli A, Longobardi L and O'Rear L (2005). Cartilage Disorders: Potential Therapeutic Use of Mesenchymal Stem Cells. In: Cianfarani S, Clemmons DR and Savage MO (eds) IGF-I and IGF binding proteins basic research and clinical management. Basel: Karger, pp. 17-30.

Spurlock ME (1997). Regulation of metabolism and growth during immune challenge: an overview of cytokine function. *J. Anim. Sci.* **75**: 1773-1783.

Sunic D, McNeil JD, Rayner TE, Andress DL and Belford DA (1998). Regulation of insulin-like growth factor-binding protein-5 by insulin-like growth factor I and interleukin-1alpha in ovine articular chondrocytes. *Endocrinology* **139**: 2356-2362.

Takaoka M, Harada H, Andl CD, Oyama K, Naomoto Y, Dempsey KL, Klein-Szanto AJ, El-Deiry WS, Grimberg A and Nakagawa H (2004). Epidermal growth factor receptor regulates aberrant expression of insulin-like growth factor-binding protein 3. *Cancer Res.* **64**: 7711-7723.

Thissen JP (2005). Regulation of Insulin-Like Growth Factor-I by Nutrition. In: Houston MS, Holly JMP and Feldman EL (eds) IGF and nutrition in health and disease. Totowa, N.J: Humana Press, pp. 25-52.

Wang P, Li N and Li JS (2002a). Mechanism of growth hormone insensitivity induced by endotoxin. *Acta Pharmacol .Sin.* **23**: 16-22.

Wang P, Li N, Li JS and Li WQ (2002b). The role of endotoxin, TNF-alpha, and IL-6 in inducing the state of growth hormone insensitivity. *World J. Gastroenterol.* **8**: 531-536.

Wang Y, Eleswarapu S, Beal WE, Swecker WS, Jr., Akers RM and Jiang H (2003). Reduced serum insulin-like growth factor (IGF) I is associated with reduced liver IGF-I mRNA and liver growth hormone receptor mRNA in food-deprived cattle. *J. Nutr.* **133**: 2555-2560.

Webel DM, Finck BN, Baker DH and Johnson RW (1997). Time course of increased plasma cytokines, cortisol, and urea nitrogen in pigs following intraperitoneal injection of lipopolysaccharide. *J. Anim. Sci.* **75**: 1514-1520.

Wright KJ, Balaji R, Hill CM, Dritz SS, Knoppel EL and Minton JE (2000). Integrated adrenal, somatotropic, and immune responses of growing pigs to treatment with lipopolysaccharide. *J. Anim. Sci.* **78**: 1892-1899.

Yakar S, Liu JL, Stannard B, Butler A, Accili D, Sauer B and LeRoith D (1999). Normal growth and development in the absence of hepatic insulin-like growth factor I. *Proc. Natl. Acad. Sci. U.S.A* **96**: 7324-7329.

Zoico E and Roubenoff R (2002). The role of cytokines in regulating protein metabolism and muscle function. *Nutr. Rev.* **60**: 39-51.

Chapter 2

Oral Inoculation with *Salmonella enterica* serovars Typhimurium or Choleraesuis Promotes Divergent Responses in the Somatotropic Growth Axis of Swine

ABSTRACT: Enteric disease and immune challenge are processes that have detrimental effects on growth performance of young swine. The current study tested the hypothesis salmonellae-induced enteric disease would perturb the endocrine growth axis in a serovar dependent fashion. Specifically, we evaluated effects of *Salmonella enterica* serovar Typhimurium (Typhimurium) and serovar Choleraesuis (Choleraesuis) on critical regulatory components of growth in young swine. Weaned pigs were housed 2 per pen with *ad libitum* access to feed and water in a 14 d experiment. Pigs were then repeatedly fed either 10^8 CFU Choleraesuis or 10^8 Typhimurium in dough balls, with control pigs receiving dough without bacteria. Bacteria were re-fed twice weekly. Rectal temperatures were monitored daily from d 0 to7 and ADFI was measured through d 14. Pigs were weighed and samples of serum were obtained for circulating IGF-I on days 0, 7, and 14. At the conclusion of the study, samples of semitendinosus muscle and liver were obtained and subsequently assayed for IGF-I, IGFBP-3, and IGFBP-5 mRNA. Rectal temperatures were elevated in pigs given Choleraesuis pigs from d 2 through d 7 ($P < 0.05$) when compared to control pigs and pigs fed Typhimurium. Pigs receiving Choleraesuis had substantially decreased feed intake on days 2, 3, 4, 7, 8, 9, and 10 ($P < 0.01$), with a trend for reduction on d 5 ($P = .08$), and they experienced an approximate 25% reduction in BW compared to control and Typhimurium pigs by the conclusion of the study. Pigs given Choleraesuis also experienced marked reductions in circulating IGF-I on d 7 ($P < 0.01$ vs. control and Typhimurium) with reductions of lesser magnitude on d 14 ($P = 0.07$ vs. control and $P < 0.05$ vs. Typhimurium). Treatment tended to affect liver IGFBP-3 mRNA ($P = 0.08$), where expression tended to be elevated in Typhimurium and Choleraesuis pigs. In contrast, IGFBP-3 mRNA relative abundance

was increased ($P < 0.03$) in pigs given Typhimurium versus control pigs. Muscle IGF-I mRNA was reduced in Choleraesuis pigs compared to control and Typhimurium ($P < 0.05$). Treatment tended to affect muscle IGFBP-3 mRNA ($P = 0.10$), where Choleraesuis had numerically less relative abundance than controls. Oral inoculation of growing pigs with Choleraesuis disrupted feed intake and BW gain, and this was accompanied by decreases in circulating IGF-I and reduced muscle expression of mRNA for IGF-I and IGFBP-3.

Keywords: IGF-I, IGFBP-3, IGFBP-5, *Salmonella*, Swine

INTRODUCTION

Enteric disease and immune challenge are processes that are generally associated with decreased growth in domestic livestock. In swine, oral infection by *Salmonella enterica* serovar Typhimirium (Typhimurium) results in growth suppression and an array of associated physiologic effects (Schwartz, 1999; Balaji et al., 2000; Jenkins et al., 2004). Infection by *Salmonella enterica* serovar Cholerasuis (Choleraesuis), another important swine pathogen, is more likely to cause septicemia in growing pigs (Schwartz, 1999), but little has been reported comparing these two serovars in terms of their effects on key regulatory elements of the endocrine growth axis. We have previously shown that infection by Typhimurium results in unmistakable effects on systemic markers of the somatotropic growth axis, namely serum IGF-I (Balaji et al., 2000; Burkey et al., 2004) and IGFBP-3 in pigs (Jenkins et al., 2004). In the current study, our objective was to directly compare the effects of oral inoculation with Typhimurium and Choleraesuis on weaned pigs. Since each serovar produces differing effects on swine, with Typhimurium producing mainly self-limiting enteritis, and Choleraesuis, a so-called swine host adapted pathogen, being more likely to result in a more serious and occasionally fatal septicemia (Schwartz, 1999). Therefore, we hypothesized that the effects of these two serovars in pigs on known promoters of growth in pigs would be differ as well.

MATERIALS AND METHODS

Experimental Design. The experimental protocol used in this study was approved by the Kansas State University Institutional Animal Care and Use Committee. The current study reports data from additional tissue collections and measurements made on from a study already published (Fraser et al., 2007). Essential details of that study and others

specific to the current report are included here for clarity. Forty-eight weaned pigs were blocked by sex and BW then randomly assigned to 1 of 3 treatments in a 14 d study. Each treatment had 8 replicates (pens) containing 2 pigs/pen. In total, 16 pigs served as negative controls, 16 pigs were repeatedly fed 10^8 CFU Typhimurium, and 16 pigs were repeatedly fed 10^8 CFU Choleraesuis. All pigs received treatments in the form of hand-fed dough balls. On d 0, pigs were fed 10^8 CFU of Choleraesuis or Typhimurium in dough balls, with control pigs receiving dough balls without bacteria. Dough balls were then re-fed on days 3, 7, and 10 resulting in animals receiving treatment twice weekly through the course of the 14 d study. Details of bacterial culture were described previously (Fraser et al., 2007). All pigs were confirmed to be negative for salmonellae organisms by fecal culture prior to inclusion in the study.

Pig BWs were recorded on days 0, 7, and 14 and feeders were weighed daily to estimate ADFI. Rectal temperatures were obtained daily from 1 pig/pen (same animal each day) from d 0 of the study until 7 d post challenge. Serum samples were also taken from 1 pig/pen (same animal each collection time) on days 0, 7, and 14 to assay for circulating IGF-I. All pigs were humanely euthanized at the conclusion of the study with tissue samples of semitendinosus muscle and liver taken for analysis of IGF-I, IGFBP-3 and IGFBP-5 mRNA expression. All tissue samples were rapidly frozen in liquid nitrogen after collection.

Serum Analysis. Serum was collected from a single pig/pen for analysis of circulating IGF-I on d 0, 7, and 14. Serum was subsequently analyzed for circulating IGF-I concentrations via an immunoradiometric assay previously described for use in swine (Balaji et al., 2000).

RNA Extraction and Reverse Transcription. Samples of muscle and liver tissue were subjected to total RNA extraction using TRI®-Reagent (Sigma Corporation, St. Louis, MO) as per the manufacturer's protocol. Following RNA isolation, removal of contaminating genomic DNA was performed with the DNA-free™ kit (Ambion Inc., Austin TX). Further quantification of isolated total RNA was done by spectrophotometry utilizing an optical density of 260 nm. Quality of total RNA was assessed by visualization of the 28S and 18S ribosomal RNA after electrophoresis of samples at 100 V for 1 hr through 1% agarose-formaldehyde gels with ethidum bromide staining. Reverse transcription was performed using Taqman® reverse transcription reagents (Applied Biosystems, Foster City, CA) and was conducted in a 50 µL final volume consisting of 25mM $MgCl_2$, 500 µM dNTP's, 2.5µM random hexamers, 0.4 U/µL RNase inhibitor, 50 U/µL MultiScribe reverse transcriptase and Taqman RT buffer. The reverse transcription mixture was then incubated at 25°C for 10 min, heated to 37°C for 60 min, and inactivated at 95°C for 5 min. Resultant cDNA was then stored at -80°C until later use.

Real Time PCR analysis for Gene Expression. Real-time quantitative PCR was used to measure the relative abundance of mRNA for IGF-I, IGFBP-3, IGFBP-5, and 18S rRNA in skeletal muscle and hepatic tissues. The PCR reactions were carried out in 96 well plates utilizing 900 n*M* of the appropriate forward and reverse primers along with 200 n*M* of the appropriate Taqman detection probe, PCR Mastermix (Applied Biosystems, Foster City, CA) and 3.5µL of the cDNA sample. The porcine specific primers and detection probes were synthesized from published GenBank sequences using

Primer Express® software (Applied Biosystems, FosterCity, CA) and were identical to those published previously (Brown et al., 2007).

Statistical Analyses. Data were analyzed utilizing the PROC MIXED procedure in SAS (SAS Inst. Inc., Cary NC). Data for RT, ADFI, BW, and serum IGF-I concentrations were analyzed as a randomized block design with repeated measures over time for each experimental unit (pens). Terms for the fixed effects of disease challenge, time, and interactions were included, with block and pen being considered random effects. Muscle and liver mRNA data were also analyzed via a PROC MIXED procedure, but without terms for repeated measures and no time statements included in the model. All means expressed are least squares means.

RESULTS

Rectal temperatures were monitored daily from d 0 through d 7 after the first bacterial feeding. There were no differences in rectal temperatures between control and Typhimurium-fed pigs the first 7 d following challenge (Figure 1). In contrast, pigs given Choleraesuis experienced dramatically increased rectal temperature starting on d 2 and continuing through the end of the monitoring period at d 7 when compared to both control and Typhimurium pigs ($P < 0.05$).

Average daily feed intake was measured by weighing feeders for each pen of pigs within each treatment daily (Figure 2), and was monitored from d 0 through the completion of the study at d 14. In general, ADFI between control pigs and pigs given Typhimurium did not differ. Conversely, Choleraesuis-challenged pigs had reduced ADFI on days 2, 3, 4, 7, 8, 9, and 10 ($P < 0.01$) and a strong tendency for reduction on d

5 ($P < 0.08$). Body weights were obtained by weighing pigs on days 0, 7, and 14 (Figure 3). Pigs were initially blocked by BW, so as expected, there were no differences in BW across treatments at d 0. However, pigs fed Choleraesuis had reduced BW on d 7 and 14 compared to control and Typhimurium pigs ($P < 0.01$).

Circulating IGF-I concentrations (Figure 4) were not different on d 0 between control, Typhimurium and Choleraesuis treatments. Moreover, serum IGF-I in control pigs and pigs receiving Typhimurium treatment remained similar through d 14. In contrast, pigs challenged with Choleraesuis had lower peripheral IGF-I concentrations on d 7 when compared to both control pigs and Typhimurium pigs ($P < 0.01$). In addition, Choleraesuis-fed pigs had reductions in serum IGF-I on d 14 when compared to controls ($P < 0.07$) and pigs given Typhimurium ($P < 0.05$).

Relative abundance of mRNA for IGF-I, IGFBP-3, and IGFBP-5 (Figure 5) in liver tissue of pigs across all treatments was variable. Only one treatment difference was observed for hepatic tissue with Typhimurium-fed pigs having greater relative abundance of IGFBP-3 mRNA compared to control pigs ($P < 0.03$). However, in the statistical model for hepatic IGFBP-3, it should be noted that the main effect of treatment only approached significance at $P = 0.08$.

For muscle tissue (Figure 6), pigs challenged with Choleraesuis had reductions in IGF-I mRNA expression when compared to control pigs ($P < 0.04$) and Typhimurium pigs ($P < 0.002$). In addition, Choleraesuis-fed pigs had reductions in IGFBP-3 mRNA expression in skeletal muscle when compared to control pigs ($P < 0.03$), although the overall treatment effect in the statistical model only tended to be significant ($P = 0.10$).

DICUSSION

Our laboratory has published findings from weaned pigs challenged with the same isolate of Typhimurium used in the current study and have noted its effects on a number of variables in pigs including known systemic mediators of growth and development (Balaji et al., 2000; Turner et al., 2002a, b; Jenkins et al., 2004). However, single oral dosages used for bacterial challenge were greater in those previous experiments, ranging from 10^9 to 10^{10} CFU. In the current experiment, we modified the approach to include an isolate of Choleraesuis to compare to Typhimurium and we utilized a lower dose of bacteria (10^8 CFU) but administered it repeatedly. This was done to more closely model fecal-oral transmission of salmonellae organisms often found in intense swine management and production conditions. In addition, we included repeated exposure to Typhimurium and Choleraesuis in an effort to create a model of disease-associated growth suppression.

In the current study, we tracked rectal temperature, ADFI, and BW as ancillary measures to document the course of treatment-induced enteric disease. Clearly, all of these measures were affected adversely by repeated oral exposure to Choleraesuis. In contrast, none of these measures were affected by repeated exposure to Typhimurium (current report and Fraser et al., 2007). Although we expected Typhimurium to disrupt ADFI and thereby potentially reduce BW, the oral dose of Typhimurium used in the study was low relative to oral doses reported to provoke enteric disease (Schwartz, 1999) and relative to those we've used previously (Balaji et al., 2000; Turner et al., 2002a,b).

We evaluated circulating IGF-I as it is widely accepted that IGF-I can be evaluated as a marker of performance and growth (Florini et al., 1996; Oksbjerg et al.,

2004; Sheffield-Moore and Urban, 2004). Furthermore, it is established that bacterial infection (Balaji et al., 2000) and other non-infectious models of sepsis (Orellana et al., 2002; Thissen et al., 2005) can lead to decreases in IGF-I in circulation, with these decreases often paralleling reductions in feed intake. In the current study, pigs challenged with Typhimurium consistently had circulating IGF-I concentrations that were very similar to those of control pigs. Although this contrasts previous findings from our group for pigs challenged with Typhimurium (Balaji et al., 2000; Jenkins et al., 2004; Turner et al., 2002a,b), the finding is consistent with a lack of Typhimurium effect on ADFI in the current study which we think is likely a consequence of the lower dose of Typhimurium used here.

In contrast to pigs receiving Typhimurium, pigs receiving Choleraesuis at this same dosage experienced marked reductions in serum IGF-I. This observation is consistent with other pathophysiologic effects of Choleraesuis observed in the current study, most notably treatment-induced reductions in ADFI. However, of potentially greater significance is our finding that, in addition to circulating IGF-I, pigs challenged with Choleraesuis experience reduced steady-state abundance of both muscle mRNA for IGF-I and for IGFBP-3. Although decreases in IGF-I mRNA in skeletal muscle have been documented after administration of LPS (Fan et al., 1994; Fernandez-Celemin et al., 2002), our findings in the current study indicate what we believe to be the first chronic disease model that has been documented to disrupt IGF-I and IGFBP-3 locally in skeletal muscle. Our working hypothesis is that these effects are most probably related to the lowered feed intakes in pigs challenged with Choleraesuis, as there is a strong body of evidence that nutrient intake plays a major role in the regulation and expression of critical

mediators of growth (Breier, 1999; Ling and Bistrian, 2005; Thissen et al., 2005). Although the effect of Choleraesuis to reduce skeletal muscle mRNA for IGFBP-3 is consistent with reduced muscle IGF-I mRNA, we caution that the overall treatment effect for IGFBP-3 mRNA only approached significance ($P = 0.10$).

Emerging dogma linking disease to mechanisms of reduced growth potential suggests that changes in feed intake, often found animals experiencing a disease challenge, are manifested by elevated peripheral pro-inflammatory cytokines (Kelley et al., 1993; Johnson, 1997) with TNF-α, IL-1, and IL-6 being predominantly implicated in altering metabolism and growth (Spurlock, 1997). It is of interest to note that in the companion study conducted with pen mates of pigs from the current study, Choleraesuis challenge was not associated with elevated circulating TNFα or IL-1β (Fraser et al., 2007). However, it is important to note that local production of inflammatory cytokines, not measured in the current study, may be of greater importance and more reflective of local regulation of skeletal muscle growth than systemic cytokines (reviewed in Gabler and Spurlock, 2008).

Although pigs challenged with repeated oral doses of Choleraesuis pigs experienced reductions in IGF-I and IGFBP-3 mRNA in skeletal muscle, these treatment effects were not found in hepatic tissue. In fact the only effect in hepatic tissues was an elevation in IGFBP-3 mRNA in pigs fed Typhimurium compared to control pigs, with pigs fed Choleraesuis being intermediate between the other treatments. The physiological significance of this isolated effect is not readily apparent especially in the context of no other treatment effects of repeated Typhimurium feeding at the dose employed in the current study. Moreover, the overall treatment effect in the statistical model, like that of

muscle IGFBP-3 mRNA, only approached significance ($P = 0.08$). So, this trend should be considered with caution.

In conclusion, data from the current study document, for the first time in pigs, both systemic and local perturbation of the IGF system in skeletal muscle associated with *Salmonella enterica* serovar Choleraesuis-induced disease. Although we expected a priori effects of Thyphimurium , at least on systemic IGF-I, the lack of effect of Typhimurium at the dose employed in the current study supports our hypothesis of servoar-specific effects on growth in pigs. Perhaps these effects reflect the more systemic nature of Choleraesuis in contrast to the more enteric nature of Typhimurium. Whether local effects on regulation of the muscle IGF system are common to other enteric diseases of swine and whether greater doses of Typhimurium would result in similar kinds of changes are intriguing questions for future research.

LITERATURE CITED

Balaji, R., K. J. Wright, C. M. Hill, S. S. Dritz, E. L. Knoppel, and J. E. Minton. 2000. Acute phase responses of pigs challenged orally with Salmonella typhimurium. J. Anim. Sci. 78:1885-1891.

Breier, B. H. 1999. Regulation of protein and energy metabolism by the somatotropic axis. Domest. Anim. Endocrinol. 17:209-218.

Brown, K.R., R. D. Goodband, M. D. Tokach, S. S. Dritz, J. L. Nelssen, J. E. Minton, J. J. Higgins, J. C. Woodworth and B. J. Johnson. 2007. Growth characteristics, blood metabolites, and insulin-like growth factor system components in maternal tissues of gilts fed L-carnitine through day seventy of gestation. J. Anim. Sci. 85:1687-1694.

Burkey, T.E., S. S. Dritz, J. C. Nietfeld, B. J. Johnson and J. E. Minton. 2004. Effect of dietary mannanoligosaccharide and sodium chlorate on the growth performance, acute-phase response, and bacterial shedding of weaned pigs challenged with *Salmonella enterica* serotype Typhimurium. J. Anim. Sci. 82:397-404.

Fan, J., P. E. Molina, M. C. Gelato, and C. H. Lang. 1994. Differential tissue regulation of insulin-like growth factor-I content and binding proteins after endotoxin. Endocrinology 134:1685-1692.

Fernandez-Celemin, L., N. Pasko, V. Blomart, and J. P. Thissen. 2002. Inhibition of muscle insulin-like growth factor I expression by tumor necrosis factor-alpha. Am. J. Physiol Endocrinol. Metab 283:E1279-E1290.

Florini, J. R., D. Z. Ewton, and S. A. Coolican. 1996. Growth hormone and the insulin-like growth factor system in myogenesis. Endocr. Rev. 17:481-517.

Fraser, J.N., B.L. Davis, K.A. Skjolaas, T.E. Burkey, S.S. Dritz, B.J. Johnson, and J.E. Minton. 2007. Effects of feeding *Salmonella enterica* serovar Typhimurium or serovar Choleraesuis to weaned pigs on growth performance and circulating insulin-like growth factor-I, tumor necrosis factor alpha, and interleukin-1 beta. J. Anim. Sci. 85:1161-1167.

Gabler, N.K. and M. E. Spurlock. 2008. Integrating the immune system with the regulation of growth and efficiency. J. Anim. Sci. 86(E. Suppl.):E64–E74.

Jenkins, N. L., J. L. Turner, S. S. Dritz, S. K. Durham, and J. E. Minton. 2004. Changes in circulating insulin-like growth factor-I, insulin-like growth factor binding proteins, and leptin in weaned pigs infected with Salmonella enterica serovar Typhimurium. Domest. Anim. Endocrinol. 26:49-60.

Johnson, R. W. 1997. Inhibition of growth by pro-inflammatory cytokines: an integrated view. J. Anim. Sci. 75:1244-1255.

Kelley, K. W., S. Kent, and R. Dantzer. 1993. Why Sick Animals Don't Grow: An Immunological Explanation. Page 119 in Growth of the pig. G. R. Hollis, ed. CAB International, Wallingford.

Ling, P. and B. R. Bistrian. 2005. Nutrition and IGF Proteins in Chronic Malnutrition and Critical Illness. Page 53 in IGF and nutrition in health and disease. M. S. Houston, J. M. P. Holly, and E. L. Feldman, eds. Humana Press, Totowa, N.J.

Oksbjerg, N., F. Gondret, and M. Vestergaard. 2004. Basic principles of muscle development and growth in meat-producing mammals as affected by the insulin-like growth factor (IGF) system. Domest. Anim. Endocrinol. 27:219-240.

Orellana, R. A., P. M. O'connor, H. V. Nguyen, J. A. Bush, A. Suryawan, M. C. Thivierge, M. L. Fiorotto, and T. A. Davis. 2002. Endotoxemia reduces skeletal muscle protein synthesis in neonates. Am. J. Physiol Endocrinol. Metab 283:E909-E916.

Schwartz, K. J. 1999. Salmonellosis. Page 535 in Diseases of swine. B. E. Straw, S. D'Allaire, W. L. Mengeling, and D. J. Taylor, eds. Iowa State University Press, Ames, Iowa.

Sheffield-Moore, M. and R. J. Urban. 2004. An overview of the endocrinology of skeletal muscle. Trends Endocrinol. Metab 15:110-115.

Spurlock, M. E. 1997. Regulation of metabolism and growth during immune challenge: an overview of cytokine function. J. Anim. Sci. 75:1773-1783.

Thissen, J. P., V. Beauloye, J. M. Ketelslegers, and L. E. Underwood. 2005. Regulation of Insulin-Like Growth Factor-I by Nutrition. Page 25 in IGF and nutrition in health and disease. M. S. Houston, J. M. P. Holly, and E. L. Feldman, eds. Humana Press, Totowa, N.J.

Turner, J. L., S. S. Dritz, J. J. Higgins, K. L. Herkelman, and J. E. Minton. 2002a. Effects of a Quillaja saponaria extract on growth performance and immune function of weanling pigs challenged with Salmonella typhimurium. J. Anim. Sci. 80:1939-1946.

Turner, J. L., S. S. Dritz, J. J. Higgins, and J. E. Minton. 2002b. Effects of Ascophyllum nodosum extract on growth performance and immune function of young pigs challenged with Salmonella typhimurium. J. Anim. Sci. 80:1947-1953.

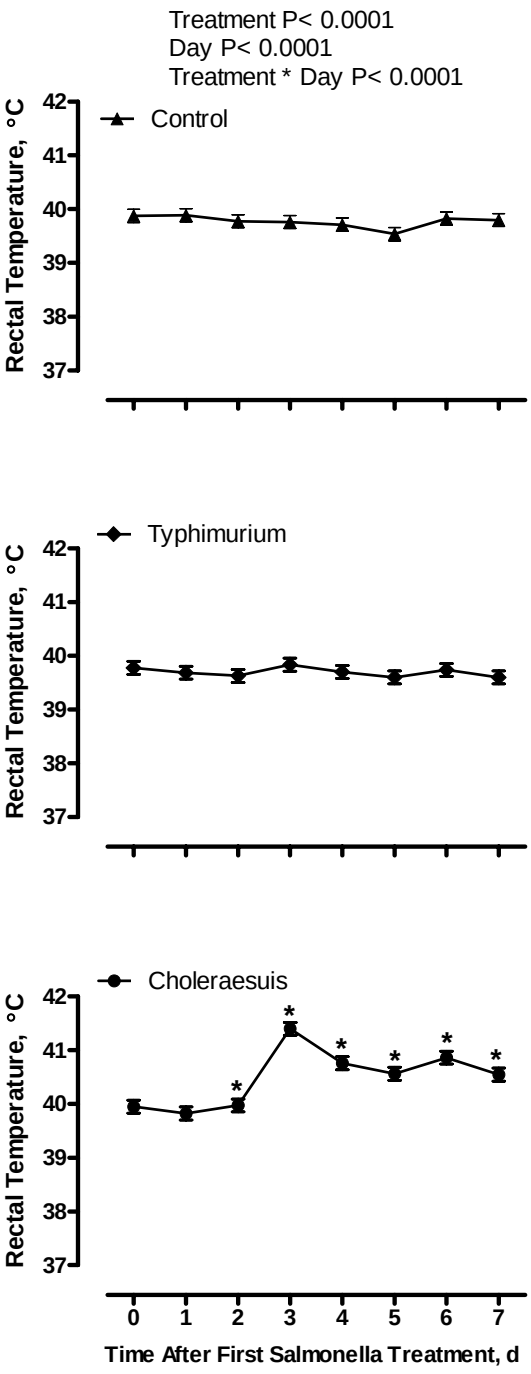

Figure 1. Rectal temperatures of pigs orally administered 10^8 CFU *Salmonella enterica* serovars Typhimurium or Cholerasuis from d 0 through d 7 after challenge. Bacteria were fed on d 0, 3, 7, and 10. Control pigs received dough balls without bacteria. Asterisks indicate days when rectal temperatures in pigs given Choleraesuis were increased relative to controls and pigs given Typhimurium ($P < 0.05$).

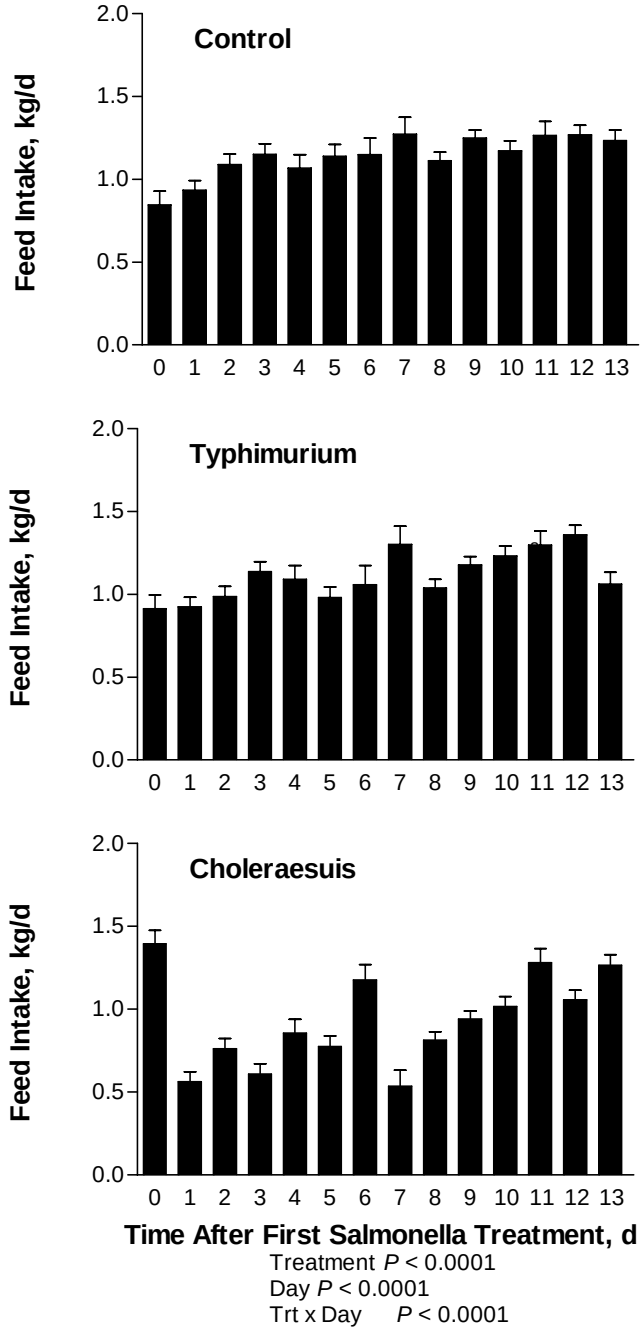

Figure 2. Average daily feed intake of control pigs and pigs orally administered 10^8 CFU *Salmonella enterica* serovars Typhimurium or Cholerasuis. Bacteria were fed on d 0, 3, 7, and 10. Control pigs received dough balls without bacteria. Pigs fed Choleraesuis experienced reductions in intake on days 2, 3, 4, 7, 8, 9, and 10 ($P < 0.01$) with a trend for reduction on d 5 ($P < 0.08$) when compared to control pigs.

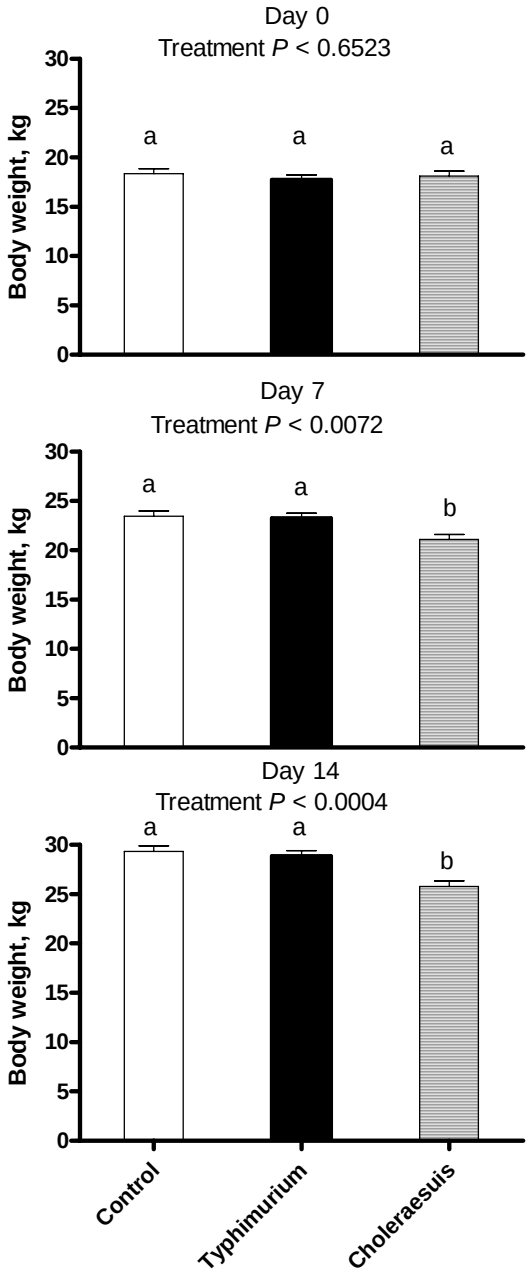

Figure 3. Body weights of control pigs and pigs orally administered 10^8 CFU *Salmonella enterica* serovars Typhimurium or Cholerasuis. Bacteria were fed on d 0, 3, 7, and 10. Control pigs received dough balls without bacteria. Pigs receiving Choleraesuis weighed less on d 7 and d 14 compared to control and pigs fed Typhimurium ($P < 0.01$).

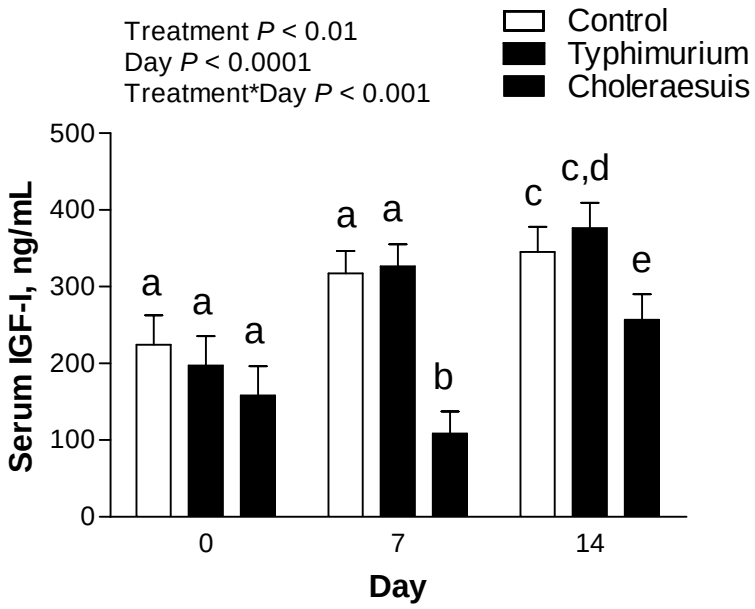

Figure 4. Serum IGF-I levels in control pigs and pigs orally administered 10^8 CFU *Salmonella enterica* serovars Typhimurium or Cholerasuis. Bacteria were fed on d 0, 3, 7, and 10. Control pigs received dough balls without bacteria. Bars without common superscript within day differ (a vs. b, $P < 0.01$); (c vs. e, $P < 0.07$); (d vs. e, $P < 0.05$).

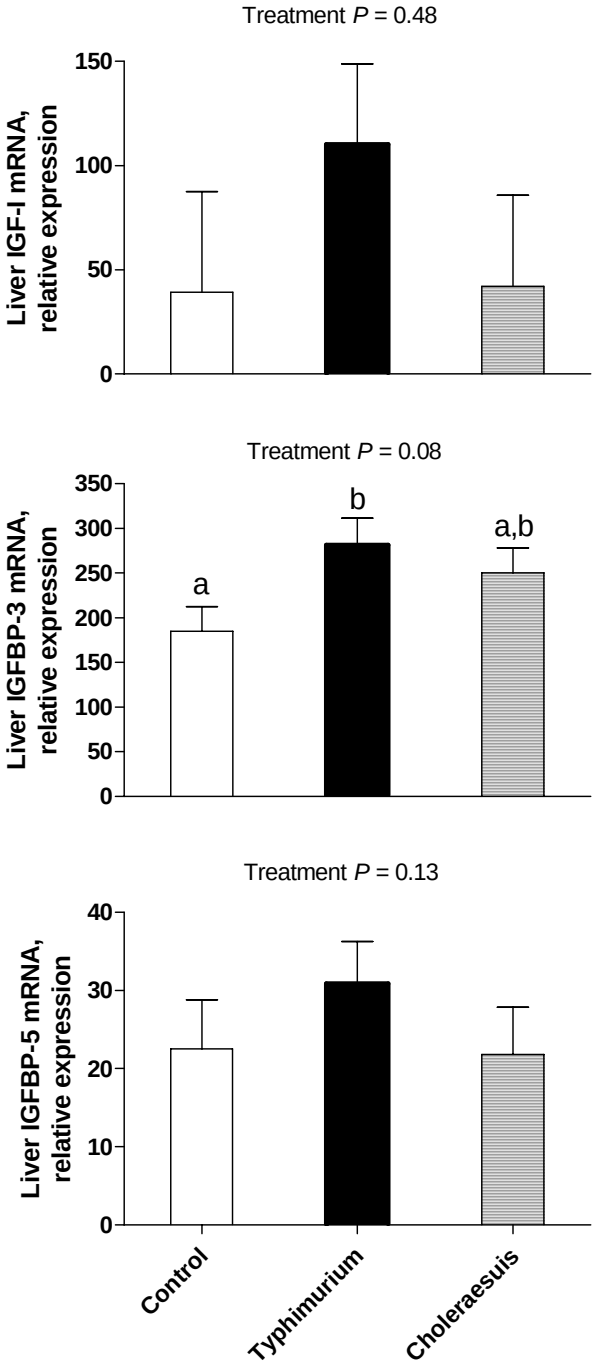

Figure 5. Expression of IGF-I, IGFBP-3 and IGFBP-5 mRNA in liver tissue of control pigs and pigs orally administered 10^8 CFU *Salmonella enterica* serovars Typhimurium or Cholerasuis. Bacteria were fed on d 0, 3, 7, and 10. Control pigs received dough balls without bacteria. Pigs were sacrificed on d 14. For liver IGFBP-3 mRNA, bars without common superscripts differ ($P < 0.03$).

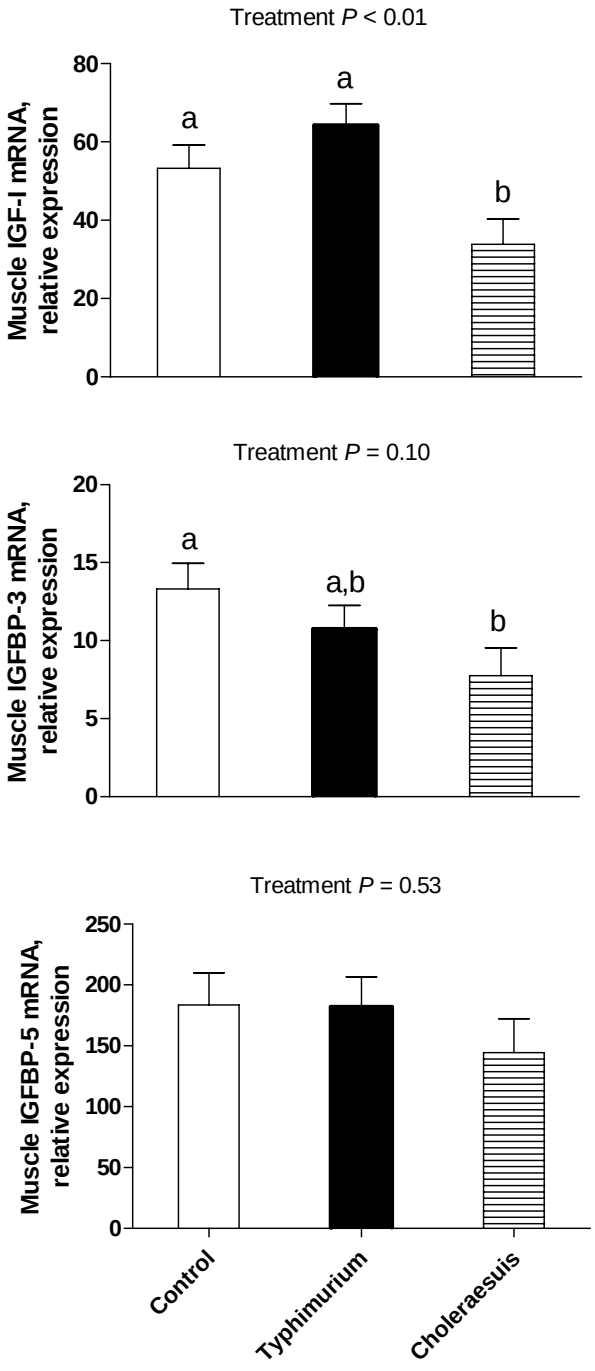

Figure 6. Expression of IGF-I, IGFBP-3 and IGFBP-5 in semitendinosus muscle tissue of control pigs and pigs orally administered 10^8 CFU *Salmonella enterica* serovars Typhimurium or Cholerasuis. Bacteria were fed on d 0, 3, 7, and 10. Control pigs received dough balls without bacteria. Pigs were sacrificed on d 14. For muscle IGF-I mRNA and IGFBP-3 mRNA, bars without common superscripts differ ($P < 0.05$).