ABSTRACT

OLIVER, WILLIAM THOMAS. Effects of growth modifiers and health status on appetite and growth regulatory mechanisms in swine. (Under the direction of Dr. Robert H. Harrell and Dr. Jack Odle)

The objective of the first experiment was to determine the effects of an anti-GnRF vaccine and porcine ST (pST) in group-housed boars and gilts. A total of 224 pigs were used in a 2x2x2 factorial design, with the factors being a GnRF vaccine (Improvac, 0 or 2 mL at 13 and 17 wk of age), pST (0 or 5 mg/d from 17 wk of age), and gender. Daily gain was increased by pST (*P*=.03) and Improvac (*P*<.001). Feed intake was lower in gilts than boars (*P*=.01), was decreased by pST (*P*=.01) and increased by Improvac treatment (*P*<.01). Lean tissue deposition rate was lower in gilts than in boars *P*<.01), increased by pST (*P*<.01) and by Improvac (*P*=.014). Fat deposition rate tended to be lower in gilts than in boars (*P*=.06), decreased by pST (*P*<.01) and increased by Improvac (*P*<.01). In conclusion, Improvac increased growth rate through increased lean and fat deposition, but concomitant use of Improvac and pST increased lean gain above either alone, while negating the increase in fat deposition in pigs treated with Improvac.

The objectives of the second experiment were to determine: 1) the effect of a high (25% fat, HF) or low (2% fat, LF) fat liquid diet on pig performance, and 2) if the limited response to exogenous pST in young pigs is dependent on the source of dietary energy. Diets were formulated to provide a constant lysine:ME ratio and were fed on a pen basis for 9 d. On d 5, pigs within a pen were randomly assigned to receive 0 or 120 μg pST•kg BW $^{-1}$•day $^{-1}$ for 4 d. Pigs gained 335±9 g/d, which resulted in an ending body weight of 7225±95 g (*P*>.42). Pigs fed the LF diet

consumed more feed than pigs fed the HF diet ($P<.01$), but calculated ME intake did not differ ($P>.20$). Pigs fed the HF diet had higher PUN concentrations than pigs fed the LF diet ($P<.01$). Treatment with pST increased circulating IGF-I ($P<.01$) and decreased PUN ($P<.01$) concentration, regardless of dietary treatment ($P>.65$). Circulating leptin was not affected by dietary treatment ($P>.26$) or pST ($P>.54$). These results demonstrate that the ST/IGF axis is responsive to exogenous pST in the young pig and the response is independent of the source of dietary energy.

The objectives of the third experiment were to determine: 1) the effect of porcine reproductive and respiratory syndrome virus (PRRSv) and *M. hyopneumoniae* (*M. hyo.*) and 2) if exogenous ST can stimulate the IGF system in health-challenged pigs. Pigs were randomly assigned to one of three treatments: 1) Non-infected, *ad libitum* intake (C); 2) Challenged with PRRSv and *M. hyo.*, *ad libitum* intake (HC); and 3) Non-infected, pair-fed to HC pigs (PF). HC pigs were infected with *M. hyo.* during jugular catheterization surgery and with PRRSv 8 d later (d 0). On d 14, pigs within a treatment group were randomly assigned to receive 0 or 120 μg pST•kg BW $^{-1}$•day $^{-1}$ for 4 d. Initial body weight did not differ ($P>.64$), but C pigs were heavier than HC and PF pigs on d 21 ($P<.01$). Disease challenge reduced feed intake beginning on d 7 of infection ($P<.04$). Basal circulating IGF-I levels averaged 322±22 ng/mL ($P>.15$) and ST administration increased circulating IGF-I ($P<.01$). Circulating leptin was not different between treatment groups ($P>.29$). The mRNA abundance of IGF-I and its receptor were not different between groups in liver or muscle ($P>.29$). These results indicate that the ST/IGF-I system was not responsible for the growth reduction found in PRRSv and M. hyo. infected pigs.

EFFECTS OF GROWTH MODIFIERS AND HEALTH STATUS ON APPETITE AND GROWTH REGULATORY MECHANISMS IN SWINE

by

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For my grandparents:

Mr. Ralph Elmo Faulkner, Sr.

Mrs. Laura Mae Christopher Faulkner

Mr. Edgar Fitch Oliver

Mrs. Sadie Hamilton Oliver

BIOGRAPHY

William Thomas Oliver was born May 5, 1974 in Burlington, NC to Forrest and Kathryn Oliver. He attended Eastern Alamance High School where he earned his diploma in June of 1992. He then attended North Carolina State University from 1992 to 1996 where he received a Bachelor of Science in Zoology in May and a Bachelor of Science in Animal Science in December. He enrolled in the Animal Science graduate program at NCSU in January 1997 under the direction of Dr. Evan "Swede" Jones. Upon Swede's retirement in January 1998, William continued his Master's program under the direction of Dr. Bob Harrell. After completing his Master's degree in December of 1999, William began his Ph.D. program by traveling to Werribbee, Australia for an internship under the direction of Dr. Frank Dunshea. Upon returning to the States in July 2000, William continued his Ph.D. program under the direction of Drs. Bob Harrell and Jack Odle.

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LITERATURE REVIEW

Components of the Somatotropin/Insulin-like Growth Factor System

Somatotropin

Somatotropin (ST) is a protein hormone containing 191 amino acids that is synthesized and secreted by the anterior pituitary gland. Release of ST is pulsatile and is regulated by the hypothalamic hormones; positively by growth hormone releasing hormone and negatively by somatostatin. Somatotropin exerts its action through binding to a specific receptor of a superfamily including receptors for the cytokines, hematopoietin as well as the ST/prolactin superfamily (Kelly et al., 1993). Upon binding the ST receptor complex dimerizes and activates a tyrosine kinase JAK2.

Insulin-Like Growth Factor System

The IGF system is composed of 2 peptide hormones of 70 and 67 amino acids for IGF-I and IGF-II, respectively (Daughaday and Rotwein, 1989). The IGF have a high degree of similarity to insulin and is highly conserved across species. The IGF have been shown to be produced by many tissues and can act by endocrine, paracrine, or an autocrine manner (Jones and Clemmons, 1995; Florini et al., 1996). The IGF-I gene structure and pattern of expression are complex systems (Adamo et al., 1991; Rotwein et al., 1993), which is consistent with its multiple physiological roles (Florini et al., 1996). Transcription of the IGF-I gene is regulated by two promoters which both contain multiple initiation sites

(Kim et al., 1991; Hall et al., 1992; Steenbergh et al., 1993). Postranscriptional events also regulate the expression of the mRNA for IGF-I (Rotwein, 1991; Hall et al., 1992). These differences in transcriptional and postranscriptional events account for several different IGF-I mRNA species.

Insulin-Like Growth Factor Binding Proteins (IGFBP). In biological fluids, the IGF are normally bound to one of six IGFBP which range from 24 to 34 kDa (Collett-Solberg and Cohen, 2000). The six IGFBP have approximately 50 % homology between themselves and 80 % homology between species (Lamson et al., 1991; Shimasaki and Ling, 1991). Most of the homology between the different IGFBP is conserved in the N- and C- terminal regions. The middle region is not highly conserved among the six IGFBP or between species (Baxter, 1994).

IGFBP-1 has a molecular weight of 25 kDa and has an RGD sequence (Ruoslahti and Pierschbacher, 1987; Shimasaki and Ling, 1991). The RGD sequence is recognized by membrane integrin receptors, which suggests IGF-independent actions of IGFBP-1 (Ruoslahti and Pierschbacher, 1987). Like IGFBP-1, IGFBP-2 (31 kDa) has an RGD sequence, but it has not exhibited binding to membrane integrin receptors (Cohen et al., 1995). The molecular weight of IGFBP-3 is 29 kDa (Gucev et al., 1997). However, IGFBP-3 has 3 glycosylation sites and is present in the circulation with a molecular weight of 40 to 44 kDa, depending on the level of glycosylation (Collett-Solberg and Cohen, 1996). IGFBP-4 is found in all biological fluids with a molecular weight of 24 (un-

glycosylated) or 28 (glycosylated) kDa (Collett-Solberg and Cohen, 2000). IGFBP-4 can be associated with the cell membrane, but is mainly found in the soluble, extracellular form (Hasegawa et al., 1995). IGFBP-5 has a molecular weight between 29 and 32 kDA, depending on glycosylation form (Collett-Solberg and Cohen, 2000). IGFBP-6 is found in its glycosylated form with a predicted molecular weight of 34 kDa. IGFBP-6 is the only binding protein that preferentially binds IGF-II rather than IGF-I (Baxter and Saunders, 1992; Claussen et al., 1995).

The discovery of IGFBP-related proteins (IGFBP-rP) has led to the proposal of a new IGFBP "superfamily" (Kim et al., 1997). IGFBP-rP share at least two similarities with IGFBP. IGFBP-rP, like IGFBP, have the highly conserved, cysteine-rich N- terminus domains (Yamanaka et al., 1997; Vorwerk et al., 1998). However, they deviate from the IGFBP structure in the midregion and C- terminus. Secondly, at least four of these IGFBP-rP bind IGF *in vitro*, although at 100-fold or lower affinity than IGFBP (Akaogi et al., 1996; Oh et al., 1996; Kim et al., 1997; Kumar et al., 1999). These findings confirm that these proteins are related to the IGFBP, but fail to fall into the classical definition of IGFBP. Thus, IGFBP-rP were classified as a subgroup with lower IGF binding affinity than IGFBP.

Acid-Labile Subunit (ALS). The majority of IGF in circulation is present as 150 kDa ternary complexes of IGF, IGFBP-3 or IGFBP-5, and ALS (Rechler, 1993; Baxter and Dai, 1994). The ALS is a 576 amino acid peptide and has an

apparent molecular weight of 84 to 86 kDa before deglycosylation and 66 kDA after (Baxter and Dai, 1994). The ALS has very low affinity for free IGF or IGFBP-3. However, it has very high affinity to IGF and IGFBP-3 as binary complexes (Twigg and Baxter, 1998). Two roles of ALS in the IGF system have been identified. First, circulation in the ternary complexes increases the half-life of IGF from about 10 min in the free form to approximately 15 hours in the complex (Guler et al., 1988; Zapf et al., 1995). Secondly, ALS prevents some of the non-specific metabolic effects of the IGF, such as hypoglycemia (Zapf et al., 1995).

Insulin-Like Growth Factor Receptors. Type 1. The type 1 IGF receptor (IGF-Ir) is a transmembrane tyrosine kinase, and is a member of the insulin receptor family. The IGF-Ir binds IGF-II and insulin in addition to IGF-I, albeit with approximately 10- and 100-fold lower affinity compared to IGF-I (Adams et al., 2000). The IGF-Ir is synthesized as a 1367 amino acid precursor (Ullrich et al., 1986). Protease cleavage results in an α chain and a β chain (Ullrich et al., 1986). The extracellular portion of the IGF-Ir includes the α chain and 195 residues of the β chain, which contains 11 and 5 potential N-linked glycosylation sites, respectively (Ullrich et al., 1986). There is a single transmembrane sequence of 23 amino acids, with the remaining 408 amino acids comprising the tyrosine kinase cytoplasmic domain. The organization of the IGF-Ir gene is similar to that of the insulin receptor gene (Abbott et al., 1992). The transcription and processing of the IGF-Ir gene results in two variants of the IGF-Ir (Yee et al.,

1989). One corresponds to the cloned cDNA sequence, and the other is explained by alternative splicing of the mRNA at the 5' end of exon 14 (Ullrich et al., 1986). The two transcripts are expressed equally in several cell types and tissues (Yee et al., 1989).

The IGF-Ir is a transmembrane tyrosine kinase and binding of a ligand results in the dimerization of receptors and activation of the tyrosine kinase activity. The close homology between the IGF-I and insulin receptors can result in the formation of hybrid dimers of insulin/IGF receptors with tyrosine kinase activity (Pessin and Frattali, 1993; Jones and Clemmons, 1995). The intracellular portion of each monomer of the insulin and IGF-I receptor contains a tyrosine kinase catalytic domain surrounded by two regulatory domains, the juxtamembrane region and a 108 residue C-tail (Sepp-Lorenzino, 1998). The juxtamembrane and C-tail domains contain the phosphotyrosine binding sites for specific cytoplasmic proteins containing SH2 (src homology 2) domains (Lowenstein et al., 1992; Kadowaki et al., 1996).

The IGF-Ir signaling cascade is an active area of research and is known to involve two major pathways (Petley et al., 1999). These pathways are activated differentially in different cell types (Petley et al., 1999). One pathway involves Shc and(or) Crk , Ras, Raf-1, the MAP kinases, and MEKK, and is associated with the proliferative response in skeletal muscle cells (Alessi et al., 1995; Coolican et al., 1997). Several of the immediate early genes, such as c-*fos* and c-*jun*, are activated by this pathway upon stimulation by IGF-I and are associated with proliferation of muscle cells (Ong et al., 1987; Hauguel-de Mouzon and

Kahn, 1991; De Meyts et al., 1995). The other pathway involves IRS-I, PI 3-kinase, and p70/p85^{s6kinase}, which is associated with the myogenic response of skeletal muscle to IGF-I (Florini et al., 1996; Coolican et al., 1997; Pinset et al., 1997).

Type 2. The type 2 IGF (IGF-IIr) receptor binds IGF-II with high affinity and is identical to the 300 kDa mannose 6-phosphate (M6P) receptor (Braulke, 1999). Fetal tissues express high levels of IGF-IIr mRNA that declines in late gestation and early postnatal periods (Sklar et al., 1989; Senior et al., 1990; Matzner et al., 1992; Sklar et al., 1992). The M6P /IGF-II receptor is involved in transport of M6P containing lysosomal enzymes and in the clearance of IGF-II from biological fluids (Braulke, 1999). There is not a consensus to whether or not the IGF-IIr is capable of cellular signaling (Minniti et al., 1992; Nishimoto, 1993; Rosenthal et al., 1994; Korner et al.,1995; Volpert et al., 1996).

Functions and Regulation of the ST/IGF Axis

The functions of the IGF are numerous. IGF-I stimulates cell growth and differentiation *in vitro* in almost all systems (Jones and Clemmons, 1995). In addition, *in vitro* experiments have been verified *in vivo*, in that IGF-I stimulates growth in both pre- and postnatal periods. Insulin-like growth factor-I has also proven to have important functions that are unrelated to somatic growth. For example, it acts synergistically with insulin as a hypoglycemic hormone in the postprandial period; this function is regulated by IGFBP-1 (Katz et al., 1997; Katz et al., 1998). In addition, IGF-I has been implicated in renal function (Hirschberg

and Kopple, 1989; Hirschberg et al., 1991; Sandra et al., 1998) and bone maintenance (Sugimoto et al., 1997). IGF-II has been implicated as an important developmental growth factor, in that high concentrations of peptide and mRNA are found *in utero* (Han et al., 1987). DeChiara et al. (1990) concluded that IGF-II is arguably the most important fetal growth factor, but has no effect on postnatal somatic growth.

The endocrine effects of IGF-I were established early (Daughaday et al., 1972), and the original somatomedin hypothesis was later revised to include the autocrine/paracrine effects of the IGFs. In 1957, Salomon and Daughaday (1957) observed that growth hormone was capable of stimulating the uptake of [35S]-sulfate into rat costal cartilage. This effect required a serum protein called sulfation factor or somatomedin, as demonstrated by the observation that serum from hypophysectomized rats with low circulating ST failed to incorporate sulfate into cartilage, while serum from ST treated, hypophysectomized rats did induce sulfate uptake. The original somatomedin hypothesis proposed by Daughaday et al. (1972) included ST effects on the liver to produce somatomedin, now known as IGF-I, which in turn acted on target tissues. The endocrine effects of IGF-I in many systems have been confirmed and extended to other species (Jones and Clemmons, 1995; Florini et al., 1996).

Challenges to the original somatomedin hypothesis came in the early 1980s, when Isaksson et al. (1985) raised the possibility that ST stimulates the local production of IGF-I by the observation that direct injection of ST into the tibial epiphysis caused tibial growth. In addition, both D'Ercole et al. (1980;

1984) and Isagaard et al. (1986) demonstrated that multiple tissues synthesize IGF-I in both the fetal and adult mouse. Further evidence for local production of IGF-I came from Russell and Spencer (1985) who injected IGF-I locally and stimulated tibial epiphyseal growth in hypophysectomized rats. In addition, Shlechter et al. (1986) abolished the stimulatory effect of ST on tibial epiphyseal cartilage by injecting both IGF-I antiserum and ST into the arterial supply of one hind limb of hypophysectomized rats. As a whole, these experiments suggest that autocrine/paracrine effects of IGF-I responding to ST were a potentially important factor in growth and development. Subsequently, the somatomedin hypothesis was revised accordingly.

The importance of the IGF system in somatic growth has been established by ablation of the genes for IGF-I and -II, as well as their receptors (Baker et al., 1993; Powell-Braxton et al., 1993). However, the most striking evidence of the autocrine/paracrine effects of IGF-I come from recent experiments using targeted, organ specific gene deletion techniques. The Cre/Lox system was used to mediate site-specific recombination by the introduction of *loxP* sites and expression of the Cre recombinase (Sjogren et al., 1999; Yakar et al., 1999). Briefly, Cre recognized two *loxP* sites flanking the fourth exon of the IGF-I gene, thus resulting in the excision of the intervening DNA. To create liver specific gene deletion, transgenic mice expressing Cre recombinase exclusively in the liver were generated, by using the control of the albumin enhancer-promoter combination (Yakar et al., 1999) or interferon-responsive promoter (Sjogren et al., 1999). Crossbreeding of the *lox-P* and Cre expressing mice resulted in

deletion of the IGF-I gene exclusively in the liver. Sjogren et al. (1999) and Yakur et al. (1999) both reduced circulating (hepatic) IGF-I concentration to below 25% of normal, while observing no changes in body weight up to 3 months and 6 weeks, respectively. In addition, they both observed an approximate four-fold increase in circulating ST. These data confirm the importance of the autocrine/paracrine effects of the IGF. Both groups question the importance of liver-derived IGF-I in postnatal growth and development.

Somatotropin (ST) regulation of the IGF system. ST deficiency, in either experimental models or disease conditions found in humans, results in reduced muscle mass, while replacement therapy with exogenous ST restores lean tissue mass (Florini et al., 1996). Different experimental models have been utilized to examine the growth-related, age-dependent effects of the ST/IGF axis. A key element in the maturation of the IGF axis is the ST regulation of the expression of IGF-I. In hypophysectomized rat model, there is no detectable expression of liver IGF-I. Replacement therapy of ST in hypophysectomized rats restored normal growth, presumably through the increase in circulating IGF-I (Bates et al., 1993).

Machlin (1972), first convincingly demonstrated that pituitary derived ST improved growth performance in swine. Since then, several studies in growing pigs have demonstrated that endogenous secretion of ST is not adequate to maximize growth in general and especially lean growth (Boyd and Bauman, 1989; NRC 1994). In general, growth rates are improved by 15-20% and feed efficiency is improved by 30 to 35% (Campbell et al., 1989; Boyd et al., 1991;

Krick et al., 1992). The improvements in performance occur by changes in the partitioning of more nutrients towards muscle accretion and less towards lipid accretion (Boyd et al., 1991). However, the response to exogenous pST in growing pigs is dependent on both age (NRC 1994; Harrell et al., 1999) and an adequate supply of dietary nutrients (Campbell et al., 1991; Krick et al., 1993).

Alterations in the IGF are thought to be the mechanism whereby ST stimulates skeletal muscle growth (Daughaday et al., 1972; Florini et al., 1996). However, whether the IGF act via endocrine stimulation or by an autocrine/paracrine mode is controversial. An endocrine mode would appear to be at least partially implicated from the higher circulating IGF-I concentrations in response to exogenous pST (Campbell et al., 1990; Coleman et al., 1994; Etherton and Bauman, 1998; Harrell et al., 1999). The higher circulating levels of IGF-I are consistent with increased hepatic expression of mRNA for IGF-I (Grant et al., 1991; Coleman et al., 1994; Brameld et al., 1996). Inconsistent changes in skeletal muscle mRNA levels of IGF-I have been found and differences may be explained by the specific muscle examined in response to pST (Grant et al., 1991; Coleman et al., 1994; Brameld et al., 1996).

Administration of exogenous pST also altered the circulating IGFBP profile in pigs (Walton and Etherton, 1989; Coleman and Etherton, 1991; Harrell et al., 1999). Exogenous pST increased IGFBP-3 and IGFBP-4 levels, but decreased circulating levels of IGFBP-2. Increased levels of IGFBP-3 are consistent with the premise that IGFBP-3 is a component of the 150 kDa complex, which provides a reservoir of circulating IGF-I.

Nutritional Regulation of the IGF system. The classic treatment utilized in determining the effects of nutrition on components of the IGF system has been the fasting model, with most studies conducted in rodents. The rodent model is unique in that dietary restriction results in a lowering of circulating ST concentrations, the opposite of what occurs in humans and domestic animals (Thissen et al., 1994). In general, circulating concentrations of IGF-I and IGFBP-3 are decreased, and concentrations of IGFBP-2 are elevated in conditions of poor nutritional status (Clemmons and Underwood, 1991; Thissen et al., 1994; Underwood et al., 1994).

Food deprivation has been studied in most species and results uniformly demonstrate that this causes a rapid decline in circulating IGF-I concentrations in rats (Straus and Takemoto, 1990; Steele et al., 1995), humans (Clemmons and Underwood, 1991; Thissen et al., 1994), pigs (Buonomo and Baile, 1991), sheep (Hodgkinson et al., 1987), and lactating dairy cows (McGuire et al., 1995). In the rat, decreased concentrations of circulating IGF-I correspond to tissue decreases in IGF-I mRNA abundance (Lowe et al., 1989; Straus and Takemoto, 1990). A 48 h fast decreased IGF-I mRNA by approximately 80% in lung and liver, 60% in the kidney, and 30 to 40% in the stomach, tests and brain, but no changes occurred in the heart (Lowe et al., 1989). Circulating concentrations of IGFBP-1 and IGFBP-2 are increased in food-deprived rats and a similar increase occurs in hepatic mRNA abundance for IGFBP-1 and IGFBP-2 (Orlowski et al., 1990; Underwood et al., 1994). Fasted neonatal pigs had decreased levels of 39 and

43 kDa IGFBP (assumed to be IGFBP-3), decreased amount of the 24 kDa IGFBP (assumed to be IGFBP-4), and increased concentrations of a 29 kDa IGFBP (McCusker et al., 1991). However, 24 hour fasting of rats and humans does not alter circulating concentrations of IGFBP-3 and liver mRNA abundance of IGFBP-3, which appear to require longer term nutritional deprivations (Donovan et al., 1991; Thissen et al., 1991).

The food deprivation model is useful in determining initial responses to nutrition, but a several day period of total absence of food does not routinely occur in domestic animal production. The specific effects of reduced energy intake in the presence of adequate levels of dietary protein have not been extensively examined and are limited only to species with monogastric digestive systems. Straus and Takemoto (1990) found that circulating IGF-I concentrations were dependent on the magnitude of energy restriction and highly correlated with liver mRNA abundance of IGF-I. Refeeding humans diets deficient in energy after a 5 day fast did not restore circulating IGF-I concentrations whereas concentrations returned to normal when subjects were refed a diet adequate in energy content (Isley et al., 1983; Isley et al., 1984).

Regulation of the IGF system by dietary protein has broad applications in domestic animal production, especially in growing pigs. Decreasing dietary intake of protein resulted in lower protein deposition rates and circulating concentrations of IGF-I in pigs (Campbell et al., 1990; Caperna et al., 1990). However, Brameld et al. (1996) did not observe any effect of dietary protein restriction on circulating concentrations of IGF-I in growing intact male pigs.

Similar reductions in circulating IGF-I have been found in protein restricted rats (Maiter et al., 1988; Fliesen et al., 1989; Thissen et al., 1990) and humans that were refed diets that differed only in protein content after a 6 day fast (Thissen et al., 1994). Also, greater IGF-I responses were observed when humans were refed high energy diets supplemented with essential amino acids compared to diets supplemented with a large proportion of nonessential amino acids (Thissen et al., 1994). The studies of fasting and then refeeding different combinations of nutrients demonstrated a high correlation between changes in nitrogen balance and concentrations of IGF-I.

Dietary protein restriction results in lower circulating IGF-I concentrations in rats by decreasing the rate of production and increasing the clearance rates (Thissen et al., 1992). The reduced production is consistent with the decreased abundance of IGF-I mRNA in cultured hepatocytes (Harp et al., 1991), liver tissue (Moats-Staats et al., 1989; Straus and Takemoto, 1990; Miura et al., 1992), and skeletal muscle (VandeHaar et al., 1991) in protein restricted rats. However, protein restriction in growing pigs altered abundance of IGF-I mRNA in adipose tissue, but not in liver or skeletal muscle (Brameld et al., 1996).

Circulating IGF-I in protein-restricted rats was preferentially bound to 40 kDa complexes as compared to the 150 kDa complex in rats fed a high protein diet (Thissen et al., 1992). In addition, Thissen et al., (Thissen et al., 1991) found that protein restricted young rats have 34% lower concentrations of circulating IGFBP-3. Underwood et al. (1994) examined expression of mRNA for the six IGFBP in liver and kidney tissue of protein restricted rats; abundance of IGFBP-1

mRNA increased in both liver and kidney, but changes in expression of the other IGFBP were tissue specific. Abundance of IGFBP-2 increased in liver, but only minimally in kidney tissue of protein restricted rats (Underwood et al., 1994). Similarly, hepatic IGFBP-2 mRNA was increased in protein restricted rats in studies by Straus and Takemoto (Straus and Takemoto, 1990). Insulin-like growth factor binding protein-3 mRNA abundance declined by 30% in liver tissue, but was not altered in the kidney (Underwood et al., 1994). Abundance of IGFBP-4 mRNA was increased in liver tissue, but was unaffected in the kidney. IGFBP-6 mRNA abundance was not altered in liver or kidney tissue (Underwood et al., 1994). Clearly in the rat, protein restriction results in tissue specific changes in mRNA for some of the IGFBP. However, this work has not been extended to other species.

Insulin regulation of the IGF system. Effects of nutrition on the IGF system could be mediated by insulin. Experimental and disease models with altered concentrations of circulating insulin were shown to be highly correlated with IGF-I status. Insulin increased IGF-I mRNA abundance and IGF-I release to the media in cultured rat hepatocytes (Phillips et al., 1991). Insulin-deficient, diabetic rats have lower hepatic IGF-I mRNA and reduced circulating IGF-I concentrations, and when supplemented with exogenous insulin concentrations of IGF-I are normalized (Bornfeldt et al., 1989; Maiter et al., 1989; Butler et al., 1996). The effect of insulin on IGF-I status is likely mediated through transcriptional control which limits studies utilizing acute supplemental insulin and

corresponding effects on glycemia (Phillips et al., 1991). This problem was circumvented by using the hyperinsulinemic-euglycemic clamp technique to examine effects on the IGF system in lactating dairy cows (McGuire et al., 1995). After a 4 d hyperinsulinemic-euglycemic clamp, circulating levels of IGF-I were twice those of the basal period. Protein restricted rats have also been shown to have reduced concentrations of insulin compared to rats fed diets that were adequate in protein content (Maiter et al., 1989). When insulin concentrations were normalized, IGF-I status was improved in rats fed adequate protein, but not in protein restricted rats.

Disease and Immunity. There are several disease conditions that are characterized by a dysfunction in the ST/IGF axis. These include insulin (Thrailkill et al., 1997) and non-insulin dependent diabetes (LeRoith et al., 1997), tumor growth (Daughaday et al., 1988), nerve injury (D'Ercole et al., 1996), acute (Goes et al., 1996) or chronic (Ding et al., 1997) renal failure, and inflammation by sepsis induced catabolism (Inoue et al., 1995). Although the endocrine system is an important regulator of immune status (Imura and Fukata, 1994; Auernhammer and Strasburger, 1995), ST deficiency or acromegaly does not result in impaired host-defense. These conditions are characterized by minor abnormalities in immune function (Rovensky et al., 1982; Kotzmann et al., 1994), but the immune system is able to compensate for these endocrine changes (Auernhammer and Strasburger, 1995), presumable through autocrine/paracrine interactions between ST, IGF-I, and cytokines (Auernhammer and Strasburger,

1995). In fact, it is becoming increasingly evident that the effects of the IGF are mediated through autocrine/paracrine means (Ohlsson et al., 2000; Yakar et al., 2000). Many cells of the immune system express IGF-I, including bone marrow stromal cells (Abboud et al., 1991) and macrophages (Fournier et al., 1995). In addition, IGF-I can act on most cells of the immune system including T- and B-lymphocytes (Stuart et al., 1991), peripheral blood mononuclear cells (Kooijman et al., 1992), and natural killer (NK) cells (Badolato et al., 1994), which express the IGF-I receptor. The presence of IGF-I and IGF-I receptor in the cells of the immune system suggests a prominent role of the IGF axis in innate as well as acquired immunity.

Insulin-like growth factor-I plays a role in innate immunity, in that IGF-I, with colony-stimulating factors such as IL-3, modulates differentiation and proliferation of myeloid lineage cells (McCubrey et al., 1991). The administration of ST or IGF-I increased the numbers of peritoneal exudative cells in mice with lethal peritonitis (Inoue et al., 1995; Inoue et al., 1996). However, the mechanisms by which IGF-I affects the number of precursor cells in the bone marrow remain largely unclear. Insulin-like growth factor-I likely affects progenitor cells directly (Clark, 1997) and indirectly by controlling the size of bones, and thus bone marrow, during growth (Kurtz et al., 1990). In addition IGF-I has anti-apoptotic effects that may enhance survival of specific myeloid lineage cells (Clark, 1997). For example IGF-I prevented apoptosis in IL-3 dependent cell lines after IL-3 withdrawal (Rodriguez-Tarduchy et al., 1992).

The interactions of immunocompetent cells with IGF-I can also affect the responsiveness of mature immune cells to antigens. Insulin-like growth factor-I enhances NK cells and macrophages and neutrophils are primed by IGF-I with respect to superoxide production (Edwards et al., 1988). In addition, endotoxin treated macrophages that were pretreated with IGF-I showed increased production of the pro-inflammatory cytokine tumor necrosis factor-α (Renier et al., 1996). *In vivo* experiments confirmed this effect, in that ST and IGF-I pretreatment produced beneficial effects on survival and bacterial killing capacity of sheep and rats challenged with infectious bacteria (Edwards et al., 1992; Inoue et al., 1995).

Insulin-like growth factor-I also affects acquired immunity by regulating lymphopoiesis before and after migration of bone marrow derived precursor cells to secondary lymphoid organs (Geffner, 1997). Insulin-like growth factor -I binds with high affinity to T- and B-cells (Tapson et al., 1988; Stuart, Meehan et al., 1991) and stimulation of T-cells with a T-cell mitogen increases IGF-I receptor numbers (Xu et al., 1995). In addition, treatment with exogenous IGF-I of dwarf mice and hypophysectomized rats resulted in increased thymus and spleen sizes (Guler et al., 1988), which reflected an increased number of resident T- and B-lymphocytes (Rosenfeld et al., 1994; Murphy et al., 1995). Similar to its role in skeletal muscle, IGF-I has a role in the differentiation of immune cells, as well as their proliferation. Insulin-like growth factor-I has been implicated in the differentiation of thymic T-cell progenitors (Gjerset et al., 1990) and bone marrow pro B-lymphocytes (Funk et al., 1994). In addition, T- and B-cells isolated from

spleen and lymph nodes of IGF-I pretreated rats were more responsive to *in vitro* activation with mitogens or antigen stimulation, respectively (Clark et al., 1993). Also, a primary antibody response was observed after immunization of IGF-I pretreated rats (Robbins et al., 1994). These data show that IGF-I has profound functional, proliferative, and differentiating effects on developing and mature lymphocytes.

Effects of endotoxin challenge on the ST/IGF system. Most studies examining the effects of a health-challenge on the regulation of the ST/IGF system utilize a challenge of the *Escherichia coli* lipopolysaccharide (LPS), which results in acute anorexia, fever, and catabolic affects on metabolism. In rats, LPS injection resulted in reduced muscle protein synthesis, which appears to be mediated through changes in translation efficiency by eukaryotic initiation factors (eIFs) such as eIF2b and eIF4E (Lang et al., 2000). In addition, administration of LPS to rats resulted in decreased circulating IGF-I and ST concentrations within 4 h (Fan et al., 1994; Soto, Martin et al., 1998). Similarly to rats, administration of LPS to humans (Lang et al., 1997), swine (Hevener et al., 1997), and sheep (Briard et al., 2000) resulted in a reduction in circulating IGF-I. However, circulating levels of ST were increased in these species. The lower circulating IGF-I levels in rats challenged with LPS are associated with decreased production of IGF-I, measured by lower mRNA abundance of liver IGF-I (Fan et al., 1994) and lower perfused liver output of IGF-I (Fan et al., 1995). Also, no differences in the plasma clearance rate of radiolabeled IGF-I were

found in rats given LPS (Fan et al., 1995). In contrast, LPS injection of rats decreased plasma levels of ALS, which could decrease the ability to form the 150 kDa circulating complex and increase the clearance rate of IGF-I (Barreca et al., 1998). The decrease in circulating ALS concentration was not attenuated by the administration of exogenous ST (Barreca et al., 1998).

Acute challenge with LPS also affects circulating IGFBP concentrations, although the effects on individual IGFBPs are species dependent. In fasted rats challenged with LPS, circulating IGFBP-1 was increased and no change was observed for circulating IGFBP-2 and –3 (Fan et al., 1994). In addition, Fan et al. (1994) observed elevated liver mRNA abundance for IGFBP-2 and –3, and an increase in liver, muscle and kidney mRNA abundance for IGFBP-1. Lang et al. (1997) observed a 5-fold increase in plasma IGFBP-1 5 hours after LPS injection in humans. Also, circulating IGFBP-2 increased, but no change was observed for IGFBP-3. A more chronic LPS challenge (8 days) decreased circulating concentrations of IGFBP-1, -2, -3, and -4 (Soto et al., 1998). In contrast, Briard et al. (2000) observed increased circulating concentrations of only IGFBP-1, while no changes were observed for IGFBP-2, - 3, or -4.

The effects on the ST/IGF system are likely mediated partially through glucocorticoids or the cytokines. An increase in circulating cortisol and tumor necrosis factor α (TNFα) was observed in pigs by 2 h after treatment with LPS (Wright et al., 2000). In addition, Li et al. (1997) observed an attenuated response of the IGF system when glucocorticoid receptor antagonists were given prior to the LPS challenge. In particular, TNFα seems to be an important

mediator of the ST/IGF response to LPS. Exogenous administration of TNFα to fasted rats decreased plasma IGF-I and ST, decreased liver and skeletal muscle IGF-I mRNA abundance, and increased circulating IGFBP-1, which are all characteristic of the response observed upon LPS challenge in rats (Fan et al., 1995). In addition, pretreatment with antibodies to TNFα attenuated the circulating IGF-I and IGFBP-1 response to LPS challenge, and completely abolished the decrease in muscle IGF-I. However, the LPS-induced changes in plasma ST and IGFBP-1 were unaffected by anti-TNFα antibodies (Fan et al., 1995). Short term (4-5 day) treatment with ST decreased the plasma TNFα response, as well as decreaseed hepatic microsomal membrane TNFα receptor content, in beef calves challenged with LPS (Elsasser et al., 1994).

Effects of Exogenous Porcine Somatotropin on Animal Performance

Effects on the sow. It is well know that the administration of bovine somatotropin (bST) routinely increases the milk output of dairy cows by 10 to 20 %, and in some situations up to 40 % (Sechen et al., 1989; Etherton and Bauman, 1998). However, bST is normally used during later lactation in dairy cows, when the cows' lactation curve is declining. In contrast, pST is normally administered when sow milk yield is increasing or peaking. The low weaning ages associated with increasing the number of pigs weaned per year per sow makes it impossible to utilize the entire milk yield curve of the sow. Because of the importance of milk yield to the growth and survival of neonatal pigs, every possible improvement in sow milk yield and composition should be investigated.

Therefore, the administration of pST to gestating (Cromwell et al., 1992; Kelley et al., 1995; Sterle et al., 1995; Sterle et al., 1996), as well as lactating (Cromwell et al., 1989; Cromwell et al., 1989; Harkins et al., 1989; Smith et al., 1991; Toner et al., 1996), sows has been investigated. However, there are conflicting reports as to whether or not exogenous pST affects lactation, let alone the nursing pig's performance. Spence et al., (1984) concluded that the administration of pST improved milk production in late lactation. In addition, Harkins et al., (1989) found that the milk yield of pST-treated pigs progressively increased in a linear manner, while milk yield of control pigs increased at a slower rate and leveled off after 8 d of treatment. The result was an improvement of about 2 kg/d of milk production. In spite of these promising data, subsequent research from different laboratories determined that sow milk yield was not improved due to exogenous pST administration (Cromwell et al., 1989; Smith et al., 1991; Cromwell et al., 1992; Toner et al., 1996). In fact, Smith et al. (1991) observed a seemingly dose dependent increase in mortality in sows treated with pST compared to controls. However, the number of experimental units in this study was insufficient to conclude that increasing the mg of pST/d increased mortality in lactating sows. In addition, Cromwell et al., (1992) also observed an increase in sow mortality when treated with pST.

In seems that the inherent level of milk production, which is largely dependent on the number and size of pigs per sow, is important in whether or not the administration of exogenous pST will increase sow milk yield. For example, Smith et al., (1991) determined that the improvement in milk yield was restricted

to the heaviest litters when pST treatments were initiated (d 7). They concluded that litters with an average piglet weight of 2.6 kg on d 7 benefited from the administration of pST. Furthermore, the large litter size used by Harkins et al., (1989) may have induced a large enough suckling response to push sow milk production close to its intrinsic limits, therefore making it possible for pST to increase milk production. In contrast, Cromwell et al., (1989) standardized litter size to 9.5 pigs per litter and found no improvement in sow milk yield or piglet performance with daily administrations of 6 mg recombinant pST.

In addition to lactation, the effect of pST on fetal survival and development has been investigated. Spence et al. (1995) observed no effect on fetal survival or survival characteristics in pregnant rats given pST during early or late gestation. Furthermore, there were no effects of pST administrations in birth weights or preweaning weight gain. However, treatment with pST did increase maternal body weight gain and serum IGF-I levels. Kelley et al. (1993) observed increased embryonic survival and survival characteristics in sows receiving twice daily injections of 15 μg/kg/BW pituitary-derived pST. In addition, piglets were observed to have increased crown rump lengths at birth and weaning (d 21). Heavier semitendinosus muscles, larger longissimus muscle cross-sectional area, longer sides, and decreased 10^{th} rib backfat were also observed in neonatal pigs whose dam was treated with pST. This indicates a larger capacity for protein deposition *in utero*. In addition, carcasses of market weight animals from pST treated sows had larger longissimus cross-sectional area, and longer carcass sides, again indicating increased muscle growth. In spite of these data,

no differences were observed in progeny from pST treated pigs in birth weight, weaning weight, or weight at slaughter, compared to pigs from control sows.

The treatment of sows with pST has not lead to the increase in performance that was hoped. The differences between the administration of ST during the increasing and decreasing portions of the lactation curve prevent the increases in production observed in dairy cows to be seen in the lactating sow (Peel and Bauman, 1987). Furthermore, the suckling response from dairy cows being machine suckled is much greater than that of pigs suckling the sow. Because of these restrictions, and the fact that dairy animals have been specifically bred for increased milk production characteristics, we should not expect the lactating sow to show the same increases in milk production.

Effects on the neonate. Piglet weaning weight is directly related to the milk yield of the sow (White et al., 1984). Milk production is directly related to the nutrient intake of the sow and nutrient intake, in turn, is influenced by the diet composition (O'Grady et al., 1985). Previous research has shown that the sow limits nutrient intake, and therefore pig's growth. Harrell et al. (1993) suggests the pig is limited by d 7-8 and Zijlstra et al. (1996) suggest sow milk production cannot support optimal baby pig growth by d 18. Therefore, limited nutrient intake by neonatal pigs results in lower weaning weights. The administration of exogenous pST could help the neonate to reach its maximum growth potential.

The effects of pST on the neonatal pig have received relatively little attention in comparison to their more mature counterparts. The results obtained by the administering of pST to neonates have been inconsistent. Harrell et al.,

(1999) removed pigs from the sow at 2 to 3 d of age and administered 120 μg/kg BW of pST daily for 4 d starting on d 10 and d 19. They found no advantages in growth rate in pigs receiving pST compared to buffer-injected controls. However, differences were observed in PUN, IGF-I, IGFBP-2, and IGFBP-3, possibly indicating that pST had the desired affects on metabolism, particularly on d 19, although they were not materialized in the performance of the animals. The effects on these metabolites were not as great in the neonate as they were in older pigs in the same study. Wester et al., (1998) also found increases in IGF-I, IGFBP-2, and IGFBP-3 in pigs weaned from the sow at 12 h to 1 d of age. The administration of pST elicited a growth response in pigs beginning at d 10. However, the levels of pST administered in this study were 10- to 15-fold (1 mg/kg BW given in 3 equal injections per d) of that which is normally given. Therefore, these data seem to indicate that the somatotrophic axis is functional in the neonatal pig, but it is not as responsive as it is in older pigs. However, no differences were observed by Dunshea et al. (1999) in growth performance or the plasma metabolites IGF-I, IGF-II, or IGFBP-3. They administered daily injections of pST (0 or 60 μg/kg BW) beginning on d 4 of lactation and continued until d 31 of lactation. In contrast to previous results, these data indicate that the somatotrophic axis is not active in the neonate, at least under these conditions. These data probably differ from Wester et al. (1998) because of the much lower injection concentration of pST. These authors used the lower dose of pST to coincide with the commercial dose of pST used in Australia, where pST is approved as a daily injection to growing and finishing pigs. Furthermore, at

growth rates of less that 300 g/d, the restraint of growth leading from restricted amount of nutrients available from the sow could cause a decreased or eradicated response to exogenous pST. In contrast, Harrell et al. (1999) showed changes in plasma metabolites, but had a growth rate of 400 to 500 g/d from d 10 to 19 d of age, irrespective of pST treatment.

Zhao et al. (1995) studied the effects of ST, as well as IGF-I, on enhancing the recovery of neonatal rats from malnutrition. Malnutrition was induced in neonatal rats by reducing maternal feed intake (60% of controls). At d 16 of lactation, pups were refed by cross fostering to dams that had not been restrictively fed. After refeeding, ST, IGF-I, and ST+IGF-I increased the growth rate of pups suffering malnutrition. By d 27, ST treated pups weighed more than pups given a placebo (saline) after refeeding. However, recovery was quicker for pups treated with ST+IGF-I or IGF-I alone. These data suggest that ST and (or) IGF-I administration after malnutrition can hasten recovery.

Effects on growing pigs. The effects of pST administration on lean growth, lipid deposition, feed efficiency and circulating metabolites seem to be age dependent (Bell et al., 1998). Therefore, this review will focus on the recently weaned pig first, then present data on older growing pigs. In general, the effects of pST on these performance and mechanistic parameters increase with the age of the pig.

Harrell et al (1997) failed to observe changes in growth performance in pigs from 10 to 25 kg. However, some circulating metabolites were affected by the administration of pST including PUN, glucose, non-esterified fatty acids

(NEFA), insulin, IGF-I and -II, and IGFBP-2. The differences seen in these metabolites were not as great as those seen in older pigs (Evock, et al., 1991; Wray-Cahen, et al., 1991; Dunshea, et al., 1992; Caperna, et al., 1993; Hansen, et al., 1997; Kerber, et al., 1998). Probably the most interesting finding in this study was the changes in PUN. PUN is an indirect estimate of amino acid oxidation, and in the case of the young growing pig, it is a measure of oxidation of dietary amino acids because available amino acids are used for muscle growth. PUN concentrations for 10 kg pigs were already relatively low (10 mg/dL), and the administration of pST maintained or decreased PUN concentrations from d 0 to d 20 of treatment while PUN for the control group increased until d 20. This resulted in pST treated pigs having lower PUN concentration by d 15 of treatment. The decrease in PUN concentrations indicate that dietary protein (amino acids) were used more efficiently for lean growth, and not used to meet the pigs energy requirement. Further evidence for this is the increase in whole body protein and protein accretion in pigs injected daily with 120 μg/kg BW pST, while whole body lipid content and lipid accretion were decreased.

Similar results have been seen in larger pigs. Krick et al. (1993) injected 150 μg/kg BW pST daily from 20 to 60 kg. PUN was the only plasma metabolite measured in this study, and increased with increasing dietary protein level (6.4 to 23.5 g/100g). As seen by Harrell et al. (1997), the administration of pST reduced PUN, and in this case, for all dietary protein levels. In contrast to 10 to 25 kg pigs, a stimulation of growth performance was detected. Average daily gain

(ADG) was increased from .88 to .96 kg/d and feed efficiency (gain/feed) was increased from .42 to .49. Average daily feed intake (ADFI) was not affected by the administration of pST. Since ADG and feed efficiency increased, and ADFI was unaffected, this indicates that the utilization of nutrients is greater for pigs treated with pST. In fact, pST treatment dramatically altered whole body composition and nutrient accretion rates. As a proportion, whole body protein was increased and whole body lipid was decreased with pST treatment. Protein and lipid accretion rates were affected in a similar manner. In addition, ash and water accretion was increased with pST treatment. These findings were also seen in earlier studies (Campbell, et al. 1988, 1990). In both studies, Campbell et al. (1988; 1990) observed improvements in all growth performance criteria and increases in the leanness of the animals.

The results of studies on younger growing pigs clearly show that the administration of exogenous pST has a greater affect than on either sows or neonatal pigs. In addition, it seems that pigs just after weaning (~10 kg) do not respond to pST as well as larger pigs (30 to 60 kg). This could be do to the still immature somatotropin/IGF axis, but is clearly evident that the effects of pST are increasing with age.

The relationships between pST treatment and energy and protein intake have been investigated. The effects of pST administration are independent of, but additive to, the effects of energy intake on growth (Campbell et al., 1988). Even at the lowest energy intake (60% *ad libitum*), pST increased ADG from .54 to .68 kg/d. These findings have been confirmed by other researchers

(Nossaman et al., 1991). However, when effects of exogenous pST were titrated by different protein levels (8.3 to 23.4 g/100g) the expected effects on growth performance were seen for the four highest protein diets, but not the two lowest protein diets (Campbell et al., 1990). The rate of protein deposition was also not affected by pST administration at the two lowest dietary protein levels. However, lipid accretion was decreased in all dietary treatment groups, albeit to a lesser extent in the lower protein groups. These results indicate that the efficacy of pST administration to improve growth is dependent on adequate dietary protein, and that protein and lipid metabolism of pigs injected with pST are independent of each other. In a study to compare energy and protein intake in pigs treated with pST and selecting between high (24 %) and low (12 %) protein diets, pST treated pigs dramatically reduced (21%) feed intake (Roberts and Azain 1997). However, the decrease in feed intake was almost exclusively limited to a lower selection of the low protein diet. This resulted in a decrease in energy intake (23 %) and no change in protein intake (g/d) in pigs treated with pST. These data indicate that pigs receiving daily injection of pST will consume less feed, and thus consume less energy, but require similar amounts of crude protein (amino acids) per day to realize the maximum benefits of pST treatment.

The majority of the research with pST has been conducted with larger pigs. Pigs at this stage of production are growing at a slower rate than younger pigs, and arguably more important, this stage of development is characterized by a decrease in protein deposition and an increase in lipid deposition. The administration of exogenous pST has marked affects on animal performance

when given to pigs in this weight range. Many researchers have shown that pigs given pST have increased weight gain while lowering their feed intake, thus improving feed efficiency (Chung et al., 1985; Etherton et al., 1986; Campbell et al., 1989; Evock et al., 1991; Evock-Clover et al., 1997). In addition, improvements in body composition are observed in pigs treated with pST (Campbell et al., 1988; Kanis et al., 1990; Smith and Kasson, 1991; Becker et al., 1992; Carter and Cromwell, 1998). Many researchers have observed a dose-dependent response in both weight gain (Etherton et al., 1986; Etherton et al., 1987; McLaren et al., 1990) and body composition (Boyd et al., 1981; Etherton et al., 1987; Evock et al., 1988; Boyd et al., 1991) improvements in pigs treated with pST. The response of animal performance seen from the administration of exogenous pST does not differ between pigs treated with pituitary- or recombinantly derived pST (Walton et al., 1986; Etherton et al., 1987; Walton et al., 1987; Evock et al., 1988; McLaren et al., 1990).

The administration of pST affects male and female pigs differently. This is to be expected because of the differences in propensity of lean gain between the genders. Campbell et al. (1990) administered daily injections of pST (100 μg/kg BW) to intact male and female pigs from 60 to 90 kg live weight and observed a 34% increase in ADG and a 84% increase in protein accretion. Porcine ST reduced feed intake by 14 %, which, in combination with increased gain, improved feed efficiency by 37%. In addition, fat deposition was decreased by 59%. However, the administration of exogenous pST had a more profound effect on gilts than on intact males. This is not surprising because females tend to

grow at a slower rate and contain less lean tissue (g/100g) compared to intact male pigs. In contrast, Hansen et al. (1997) observed improvements in performance of barrows administered pST (4 mg/d) that compared to females receiving pST in the study by Campbell et al. (1990). Barrows eat more feed and typically accrue more fat than intact boars, thus leading to a greater improvement upon treatment with exogenous pST compared to boars. However, Hansen et al. (1997) noted that most of the increase in ADG was from an increased visceral organ weight. Only 17 % of the total increase in ADG were attributable to an increase in empty body weight. This is in agreement with other researchers (Klindt et al., 1992). In spite of organ weight increasing to a larger extent than empty carcass weight, the increase in protein accretion and whole body protein of pigs treated with pST make the empty carcass more valuable than pigs not treated.

The treatment of growing pigs with pST can eliminate the inherent differences seen in growth of boars, barrows, and gilts. Untreated, boars will consume less feed, have a faster rate of gain, contain more fat, and produce more protein and water than gilts. The performance of barrows will fall between gilts and boars. Exogenous pST treatment will affect barrows to a larger degree than boars, and will affect gilts to a larger degree than barrows. Campbell et al. (1989) increased growth rate with daily injections (100μg/kg BW) of exogenous pST by 10, 16, and 22% and feed efficiency by 19, 32, and 34% in boars, barrows, and gilts respectively. In addition, body fat content was reduced by 22, 33, and 36% with a corresponding increase in body protein and water content for

boars, barrows, and gilts, respectively, in pST treated pigs. This study clearly shows the differences in the effects of the administration of pST to boars, barrows, and gilts, in that the inherent differences in growth and lipid accretion were not detected in pST treated pigs.

The interaction of genotype and pST treatment has been investigated exhaustively. The treatment with exogenous pST has increased pig performance to a lesser degree in pigs with a greater capacity for lean tissue gain (Kanis et al., 1990; Verstegen et al., 1990; Bidanel et al., 1991; Klindt et al., 1992; Buonomo et al., 1995; Hansen et al., 1997). Since pST has profound affects lipid metabolism, this is an expected response. Klindt et al. (1992) observed greater changes in ADG and lean accretion rate in obese compared to lean pigs with the administration of pST (2 and 4 mg/d) by sustained-release implant. Kanis et al. (1990) observed a larger response in lean tissue growth to pST treatment in obese pigs compared to lean pigs from 60 to 100 kg, although from 100 to 140 kg, leaner pigs responded to a larger degree to pST treatment. This could be simply due to the decreasing propensity for lean tissue growth for all genotypes at this stage of development. In contrast, some authors have not observed differences in different genotypes of pigs administered pST (Campbell et al., 1990; Nossaman et al., 1991; Klindt et al., 1995). Campbell et al. (1990) administered daily injections of pST (1 mg/kg BW) to slow and fast growing pigs and observed a profound effect on ADG, ADFI, and composition of gain. However, no differences were observed between pigs selected for slow or fast growth rates. In addition, Nossaman et al. (1991) found no differences in pigs

with a greater genetic potential for lipid gain. In general, pigs with a higher predisposition for fatness will benefit from the administration of exogenous pST. Although some investigators have not observed these genotype differences, the effect of pST on lipid metabolism dictates that pigs with more adipose tissue will be affected to a greater extent by pST administration.

Porcine reproductive and respiratory syndrome virus (PRRSv) and *Mycoplasma hyopneumoniae* (*M. hyopneumoniae*).

Porcine respiratory disease complex (PRDC) is now considered a serious health problem in the swine industry, both in the United States and abroad. The pneumonia associated with PRDC is due to a combination of several viral and bacterial pathogens. PRRSv and *M. hyopneumoniae* are two of the most commonly isolated agents from pigs with clinical signs of PRDC and are now considered two of the most important causative agents.

PRRSv.

Porcine reproductive and respiratory syndrome virus, the causative agent of porcine reproductive and respiratory syndrome (PRRS), was first isolated from pulmonary alveolar macrophages (PAMs) in the Netherlands by Wensvoort et al. (1991), and was designated at the time as Lelystad virus (LV). Subsequent isolation and characterization of PRRSv occurred in the United States from the continuous cell line ATCC CL2621 (Benfield et al., 1992; Collins et al., 1992). Porcine reproductive and respiratory syndrome virus was found to be a small, enveloped positive-stranded RNA virus, similar to equine arthritis virus (EAV),

lactate dehydrogenase-elevating virus (LDV), and simian hemorrhagic fever virus, thus belonging to the *Arteriviridae* family. Porcine reproductive and respiratory syndrome virus has been documented throughout the world and remains an economically important disease (Rossow, 1998). All PRRSv strains identified cause similar diseases, but two distinct genotypes of the PRRSv have been identified, designated as the European and North American PRRSv (Meng et al., 1995). In addition, recent evidence suggests, as a consequence of quasispecies evolution (Rowland et al., 1999) and RNA recombination (Yuan et al., 1999), that many subspecies, or variants, of both the European and North American PRRSv exist. The heterogeneity caused by quasispecies evolution and RNA recombination could be a factor in the persistency observed in PRRSv infected herds.

Monocytes, especially PAMs, are the primary permissive targets of PRRSv (Rossow et al., 1996; Molitor et al., 1997). Duan et al. (1998) confirmed that virus entry into PAMs occurs by receptor mediated endocytosis and a PRRSv receptor was identified by generation of PAM-specific monoclonal antibodies. Two antibodies were generated that prevented PRRSv infection by binding to a 210 kDa PAM protein, which is presumably used for virus attachment. After binding to the cell, virus particles are internalized in clathrin-like coated vesicles via a microfilament-dependent process (Kreutz and Ackermann, 1996; Nauwynch et al., 1999). At three hours post-infection, the first signs of degeneration were observed, and viral antigen was detected in the cytoplasm six hours after infection (Nauwynch et al., 1999). To examine the

synthesis and processing of the PRRSv structural proteins, Mardassi et al., (1996) used monospecific antisera against the nucleocapsid (N), integral membrane (M), and envelope (E) proteins, and observed disulfide linked M-E heterodimers that are incorporated into virions preceding viral budding. Accumulation of enveloped particles is observed between the smooth endoplasmic reticulum and golgi apparatus (Suarez, 2000), and virus assembly is completed by interaction with the N protein that has accumulated in the cytosol.

Infection of PAMs with PRRSv in vitro causes rapid apoptosis (Bloemraad et al., 1994; Patonetal, 1992). The gp5 protein of PRRSv, an N-glycosylated structural protein (Meulenberg et al., 1995; 1996), is capable of inducing apoptosis in PAMs *in vitro* (Suarez et al., 1996). The gp5-induced apoptosis could not be prevented by bcl-2 expression, which suggests that gp5 may act downstream in the apoptosis cascade (Suarez, 1996). In a study with two different North American PRRSv, bystander cells that were unaffected by the virus were observed to undergo apoptosis (Sirinarumitr et al., 1998). Oleksiewicz et al. (1999) observed a 40% reduction in phagocytozing cells after infection of macrophages with European PRRSv *in vitro*. In addition, they observed a reduced phagocytic ability of infected macrophages, but uninfected macrophages were unaffected. This finding suggests the lack of soluble suppressive factors affecting bystander cells, contradicting the observations of Sirinarumitr et al., (1998).

There are at least five sub-populations of PAMs with differing susceptibility to PRRSv infection, and these sub-populations vary with the age and disease status of the pig (Duan et al., 1998). The changes in sub-populations may affect the overall response of the pig to a viral challenge. A higher percentage of PRRSv sensitive PAMs are present in young pigs (Shibata et al., 1997), and there is a higher PRRSv replication in these pigs (Mengeling et al., 1995; Shibata et al., 1997). As a consequence, younger pigs are more susceptible to the respiratory form of PRRS.

In addition to macrophages, PRRSv has been reported to influence T-cells, although some observations are contradictory. Both Christianson et al., (1993) and Shimizu et al., (1996) observed increases in $CD2^+$ and $CD8^+$ T-cells, and a decrease in $CD4^+$ T-cells. The changes in $CD8^+$ cells, a marker for cytotoxic T-cells, peaked on d 28 to 35 post-infection (Shimizu et al., 1996), while the decline in $CD4^+$ cells, including T-helper cells, lasted at least 14 days (Christianson et al. 1993; Shimizu et al., 1996). However, proliferation of these cells was not observed during *in vitro* stimulation, suggesting an indirect effect of PRRSv (Shimizu et al., 1996). The observations of Christianson et al. (1993) and Shimizu et al. (1996) were directly contradictory to Zhou et al. (1992), who observed an increase in $CD4^+$ cells and a decrease in $CD8^+$ cells, respectively. One explanation of this discrepancy was the variation in pathogenicity among PRRSv variants between these experiments. As yet, the mechanism by which PRRSv alters the population of T-cells in unknown, but many theories have been

proposed, including $CD4^+$ cell death and $CD8^+$ cell stimulation. Alternatively, PRRSv may act at the point of intrathymic T-cell differentiation.

B-Cells have also been observed to be affected by PRRSv. The proliferation of B-Cells from peripheral blood lymphocytes was diminished in PRRSv infected piglets (Vezina et al., 1996), although this response was transient. However, PRRS is characterized by a persistent infection of PRRSv in the presence of a strong humoral immune response. The immune response to individual viral proteins has been examined, and the antibody response is mounted primarily to the nucleocapsid, with lesser responses observed to the matrix protein and the major envelope protein (Loemba et al., 1996; Meulenberg et al., 1995). Immunoglobulin (Ig) M concentrations are detectable seven days after infection, peak at 14 to 21 d post-infection, and decline to undetectable levels by 35 to 42 days, while IgG levels peak at 21 to 28 d post-infection and are measurable for several months thereafter. Albina et al. (1998) also observed a stimulation of the immune system, in that IgM, $CD2^+$ and $CD8^+$ cells, as well as total WBC counts were increased after PRRSv infected pigs were infected with pseudorabies virus.

PRRSv has been documented to alter the production of pro-inflammatory cytokines, an important immune function of cells of the monocyte/macrophage lineage. Virus infected cells commonly produced interferons (IFN), and IFN-α has been observed to be capable of inhibiting the growth of PRRSv *in vitro* (Albina et al., 1998). However, Albina et al. (1998) observed low concentrations of circulating IFNα in PRRSv infected pigs and IFN-α was undetectable in lung

secretions. In addition, super-infection of PAMs with a good stimulator of IFN-α, transmissible gastroenteritis virus, did not induce IFNα production in PRRSv pigs. These findings led to the notion that IFN-α production may be down regulated following infection with PRRSv.

Similar to previous reports, Lopez-Fuertez et al. (2000) observed an IFN-α induced inhibition of PRRSv replication *in vitro*. In the same experiment, tumor necrosis factor (TNF) -α also decreased virus replication. However, the addition of TNF-α to PAM cultures containing IFN-α did not further reduce virus replication, although TNF-α has been shown to synergize with INFs in other virus models (Wong and Goeddel, 1986; Biron, 1998). Although PRRSv infected PAMs did not produce TNF-α (Asai et al., 1999; Lopez-Fuertez et al., 2000), Lopez-Fuertez et al. (2000) reported a 46 and 30 % reduction, at 2 and 6 h respectively, of phorbol myristate acetate (PMA)-induced expression of TNF-α mRNA in PRRSv infected cells compared to PMA stimulated cells. Interleukin (IL) -1 mRNA expression was similarly decreased in PRRSv infected cells at 6 h post-infection, but not at 2 h, compared to PMA stimulated cells. Asai et al. (1999) also observed an increase in IL-6 in PRRSv infected pigs by seven days after infection. Interleukin-6 concentrations peaked at 14 d post-infection, were still elevated at d 21, but were undetectable at the termination of the experiment on d 28. In addition haptoglobin concentrations increased with PRRSv infection, peaked seven days post-infection, and decreased to undetectable levels at d 28.

In young pigs, PRSSv induces fever, anorexia, vomiting, and coughing (Benfield et al., 1992). In an experimental PRRS model, PRRSv infection

produced only transient (8 d) reductions in average daily gain (~50% on d 8) and feed intake (~20% on d 8; Greiner et al., 1999, 2000). However, under field conditions, PRRS produces a much more chronic depression in animal performance, presumably due to its persistence in a herd (Blaha, 2000) and interactions with other pathogens (Zenan, 1994). Williams et al. (1997) observed that chronic pathogen exposure under commercial conditions results in reduced growth rate, feed intake, and utilization of dietary nutrients.

M. hyopneumoniae.

M. hyopneumoniae is the causative agent of mycoplasmal pneumonia of swine (MPS), also called enzootic pneumonia, and is one of the most common and economically important diseases in the swine industry. The incubation period of MPS is a matter of debate. Ross et al. (1999) reported the incubation period of MPS to be 10 to 16 d under natural conditions. However, in experimentally induced infection, considerable variation may occur. Sorensen et al. (1997) noted that coughing was present in most pigs only six d after infection and *M. hyopneumoniae* was detected on d seven, while Morris et al. (1995) first detected seroconversion of a herd three wk after exposure to *M. hyopneumoniae.*

Upon exposure, *M. hyopneumoniae* adheres to cells of the respiratory tract, specifically to the cilia of ciliated cells and an extensive loss of cilia is observed (Blanchard et al., 1992; Mebos et al., 1977). The adherence to these ciliated cells is required for colonization of the agent on the respiratory tract and the development of pneumonia, which is a receptor-mediated process (Zhang et

al., 1994; Zielinski and Ross, 1993). Specifically, a sulfated glycolipid receptor on the cilia of the respiratory cells serves as a receptor for *M. hyopneumoniae* (Zhang et al., (1994a,b). Several adhesion proteins have been discovered for many pathogenic bacteria, and Zhang et al. (1995) used monoclonal antibodies against several antigens of *M. hyopneumoniae* and identified a 97 kDa (P97) adhesion protein. Semi-purified P97 was able to bind to cilia and blocked the adherence of *M. hyopneumoniae* to the ciliated cells. Additionally, Chen et al. (1998) identified a 110 kDa adhesion-like glycoprotein (P110) consisting of two subunits (54 and 28 kDa). Similarly to P97, purified P110 inhibited the adherence of *M. hyopneumoniae* to pulminary epithelial cells.

The pattern of transmission of *M. hyopneumoniae* has been reported to be due to direct contact, particularly from older to younger pigs. Seroconversion against *M. hyopneumoniae* was seven times more likely if pigs were in direct contact with other infected pigs (Morris et al., 1995). In addition, Chen et al. (1998) noted that spread of *M. hyopneumoniae* is primarily through nose to nose contact, but observed that aerosol transmission was possible at high stocking density and infection levels. In fact, Stärk et al. (1998) isolated *M. hyopneumoniae* from air samples taken from finishing houses holding infected pigs, and positive samples were attained over pens that did not contain coughing pigs, implying that *M. hyopneumoniae* is able to diffuse through the house. However, most authors agree the most frequent route of transmission is through direct contact.

A humoral immune response to *M. hyopneumoniae* probably plays an important role in the host defense against MPS (Blanchard et al., 1992). However, Wallgreen et al. (1998) observed clinical signs of mycoplasmosis in sows during an outbreak of the disease, despite the sows being previously seropositive. Thus, the protection against *M. hyopneumoniae* seems to be dependent on the balance between the immune status of the animal and the pathogen load. Protective immunity was transferred from sow to piglet, as no clinical signs of disease developed among pigs nursing infected sows (Wallgreen et al., 1998). However, the expected decline in maternal immunity was observed in these piglets. Respiratory disease was recorded only in pigs older than three wk, and antibodies to *M. hyopneumoniae* were not detected in coughing animals between four to seven wk of age (Wallgreen et al., 1998). This finding indicates a loss of maternal immunity and an inability of the pigs to mount their own immune response to *M. hyopneumoniae*. The lack of a humoral immune response in young pigs has been observed (Metzeger et al., 1978), although an ability to produce antibodies to intramuscular injection of flagellin *in utero* has been demonstrated (Tlaskalova-Hogenova et al., 1994). Still, the lack of serum antibodies to *M. hyopneumoniae* is likely due to to poor immune function. Wallgreen et al. (1998) determined an obvious age-dependent ability of peripheral blood mononuclear cells (PBMC) to produce antibodies against *M. hyopneumoniae in vitro*, with PBMC from five to nine wk of age beginning to have the ability to produce antibodies. Similar to Wallgreen et al. (1998), Thacker et al. (2000) failed to detect *M. hyopneumoniae* specific antibodies in the

bronchioalveolar lavage fluid (BALF) or serum of boars infected at 7 to 10 d of age. However, boars vaccinated with a mycoplasma bacterin generated antibodies (IgG and IgA) against *M. hyopneumoniae*. In a survey to determine the incidence of infection with *M. hyopneumoniae* on a commercial farrow-to-finish farm, 98% of pigs were seropositive at 144 d of age, while none had been positive at 30, 59, and 86 d of age (Sheldrake et al., 1990). Similarly, Leon et al. (2001) studied three farrow-to-finish farms to estimate the incidence of *M. hyopneumoniae* infection and found that most pigs were seronegative at 27, 70, and 94 d of age, suggesting that no circulation of *M. hyopneumoniae* occurred during the growing phase of production. However, a high proportion of pigs seroconverted between 94 and 125 d of age, suggesting that the transfer of animals into the finishing houses is the critical period for *M. hyopneumoniae* infection on these farms.

M. hyopneumoniae interacts with alveolar macrophages to produce an inflammatory cytokine response (Asai et al., 1993). Asai et al. (1993) observed that TNF-α, IL-1, and IL-6 were increased in BALF after infection with *M. hyopneumoniae*. Thacker et al. (2000) also observed an increase in TNF-α in BALF after infection. In addition, Okada et al. (2000) observed a reduced number of macrophages, neutrophils and lymphocytes, as well as decreased levels of TNF-α in BALF of pigs immunized with a *M. hyopneumoniae* inactivated vaccine. In contrast, Thanawongnuwech et al. (2001) only observed a trend for IL-1 and IL-8 to be increased, with no increase in TNF-α in an *in vitro* co-culture of tracheal ring explants and PAMs from *M. hyopneumoniae* infected pigs. The

cause of the increase in IL-8 in this study is not evident, although the authors speculate it may be an artifact due to tissue damage during the harvesting of tracheal rings.

Pigs infected with *M. hyopneumoniae* gain weight at a slower rate, eat less feed, and have a poorer feed conversion, and MPS is described as chronic disease with a high morbidity and a low mortality. Although ADG is routinely reduced by *M. hyopneumoniae* infection, Straw et al. (1989) reported a tremendous variation with the reduction in gain due to MPS, which ranged from 2.8 to 44.1 % over 24 different studies. However, much of this variation could be attributed to the scoring of lung lesions (Morrison et al., 1986), in that lung lesions resolve with time and results associated with these lesions will depend on the time of infection relative to the time at slaughter (Noyes et al., 1990; Wallgreen et al., 1993). Nonetheless, *M. hyopneumoniae* infection reduced pig performance in numerous studies. Pointon et al. (1985) observed a 15.9 % reduction in growth rate in pigs with MPS between 8 to 85 kg. In addition, feed utilization was 13.8% less efficient in 10 to 25 kg pigs with MPS. Rautianen et al. (2000) observed a 224g difference in ADG between *M. hyopneumoniae* infected and control pigs after adjusting for herd, pen, weight, and sex.

Potentiation by PRRSv and M. hyopneumoniae. The observation that many secondary agents, both bacterial and viral, have been isolated from PRRSv infected herds has led to the postulation of interactions between PRRSv and these other agents. This apparent increase in susceptibility to secondary agents suggests PRRSv is an immunosuppresive virus. Groschup et al. (1993)

observed significant associations between PRRSv and swine influenza virus, as well as porcine respiratory coronavirus. They postulated that an increased severity of disease could be caused by PRRSv promotion of these secondary agents, synergism between PRRSv, or external factors potentiating both agents. A reduction in the population of MAPs due to PRRSv infection was suggested to be the cause of the increased susceptibility to secondary agents. In addition to viruses, bacterial agents have been implicated as synergistic agents associated with PRRSv (Galina et al., 1994). Zeman et al. (1996) confirmed bacterial infections in 58% of 221 PRRS cases. However, Cooper et al. (1995) observed that PRRSv did not potentiate bacterial pathogens, specifically *Haemophilus parasuis*, *Streptococcus suis*, *Salmonella cholerasuis*, and *Pasteurella multocida*. Van Alstine et al. (1996) observed similar results, in that PRRSv infection did not increase the severity of experimental *M. hyopneumoniae* infection. No differences were observed in average days coughing, gross pneumonic lung scores, or microscopic lung lesion scores. However, growth performance was not reported in this study. Taken together, there are contradictory data as to whether or not PRRSv potentiates secondary infections, but the evidence that suggests a potentiation is largely circumstantial. In experiments designed to specifically address this question, PRRSv did not exacerbate secondary infections. Thus, it appears unlikely that PRRSv potentiates a *M. hyopneumoniae* infection.

In contrast to the lack of effect of PRRSv indicated above, *M. hyopneumoniae* seems to potentiate the effects of PRRSv infections. Thacker et

al. (1999) reported that PRRSv induced pneumonia lesions were more severe and persistent in *M. hyopneumoniae* infected pigs. At 28 or 38 d after infection with PRRSv, *M. hyopneumoniae* infected pigs still had PRRSv induced lesions, while pigs infected with PRRSv alone were normal. In addition, *M. hyopneumoniae* infected pigs with minimal mycoplasmal pneumonia lesions had more severe PRRSv induced pneumonia lesions than pigs infected with PRRSv alone. These results are similar to previous findings for other mycoplasmas that have been implicated in playing a synergistic role with human immunodeficiency virus (Baseman and Tully, 1997; Blanchard and Montagnier, 1994). In a follow up study, Thacker et al., (2000) observed that vaccination against PRRSv did not decrease the potentiation of PRRSv by *M. hyopneumoniae*, while no potentiation was reported when pigs were vaccinated with *M. hyopneumoniae* bacterin. In addition, dual infection with both PRRSv and *M. hyopneumoniae* increased the production of IL1, IL8, and TNF-α compared to either infection alone (Thanawongnuwech et al., 2001), which suggests that cytokine production may play an important role in the chronic pneumonia induced by the simultaneous infection by both agents.

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CHAPTER 2

A GnRF VACCINE (IMPROVAC®) AND PORCINE SOMATOTROPIN HAVE SYNERGISTIC AND ADDITIVE EFFECTS ON GROWTH PERFORMANCE IN GROUP-HOUSED BOARS AND GILTS

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ABSTRACT

Two hundred and twenty four pigs (112 boars, 112 gilts) housed in pens of 7 pigs/pen were used in a 2 x 2 x 2 factorial design, with the factors being vaccination with a GnRF vaccine, Improvac (0 or 2 mL at 13 and 17 wk of age), porcine somatotropin (pST; 0 or 5 mg/d from 17 wk of age), and gender. Pigs were weighed and feed disappearance was measured from 17 wk of age until slaughter at 21 wk of age. Body composition was estimated by dual-energy X-ray absorptiometry in 2 focus pigs per pen at 17 and 21 wk of age. Testes and ovary weights at slaughter were decreased by Improvac treatment ($P < .001$) but were not altered by pST treatment ($P > .44$). Daily gain was lower for gilts than boars (1128 *v.* 1299 g/d, $P < .001$), and was increased by pST (1172 *v.* 1255 g/d, $P = .003$) and Improvac (1150 *v.* 1276 g/d, $P < .001$) treatments. Feed intake was lower in gilts than boars (2774 *v.* 3033 g/d, $P = .002$), was decreased by pST (3037 *v.* 2770 g/d, $P = .002$) and increased by Improvac treatment (2702 *v.* 3105 g/d, $P < .001$). As a result of the differences in feed intake and daily gain, feed conversion efficiency (gain:feed) was lower for gilts than for boars (0.403 *v.* 0.427 $P = .025$), was improved by pST (0.385 *v.* 0.452, $P < .001$), but unchanged by Improvac treatment (0.423 *v.* 0.410, $P = .22$). Carcass weight was lower in gilts than in boars (75.3 *v.* 77.0 kg, $P = .012$), was unchanged by pST treatment (75.9 *v.* 76.4 kg, $P = .40$) and increased by Improvac treatment (75.1 *v.* 77.2 kg, $P = 0.003$). Lean tissue deposition rate was lower in gilts than in boars (579 *v.* 725 g/d, $P < .001$), increased by pST (609 *v.* 696 g/d, $P < .001$) and by Improvac treatment (623 *v.* 682 g/d, $P = .014$). Fat deposition rate tended to be lower in gilts than in boars

(214 *v.* 247 g/d, $P = .063$), decreased by pST treatment (263 *v.* 198 g/d, $P < .001$) and increased by Improvac treatment (197 *v.* 264 g/d, $P < .001$). For pigs treated with both pST and Improvac, daily gain and lean tissue deposition rate was greater than pigs that received either treatment alone while fat deposition rate and feed intake were similar to untreated control pigs. In conclusion, Improvac increased growth rate through increased lean and fat deposition, but concomitant use of Improvac and pST increased lean gain above either alone, while negating the increase in fat deposition in pigs treated with Improvac.

Key words: GnRF, pST, Swine

INTRODUCTION

Boar taint results from the accumulation of chemical agents, primarily skatole and androstenone, in the adipose tissue of mature, intact male pigs (Bonneau, 1982). To negate the undesirable accumulation of these substances male pigs are routinely castrated in the United States, which requires labor and introduces stress and the risk of complications from the surgery to the pigs. More importantly, barrows consume more feed, grow at a slower rate, and will have proportionally more fat and less muscle compared to boars (Campbell et al., 1989). An alternative to castration in the traditional manner is immunocastration, which is based on immunizing the animals against a hormone that is critical for normal sexual development and maintenance. The efficacy of anti-GnRH vaccines has been well established in bulls and heifers (Adams et al., 1993; Prendiville et al., 1995; Huxsoll et al., 1998) and an anti-GnRH vaccine

(Improvac; CSL Limited) has recently been shown to be effective in suppressing sexual characteristics in boars (Dunshea et al., 2001) and gilts (McCauley et al., 2002). Unexpectedly, a positive growth response was observed in boars and gilts vaccinated against GnRH, but it is unknown whether metabolic or behavioral changes elicits this response.

Porcine somatotropin (pST) has been utilized effectively to alter the partitioning of energy in feed away from fat and towards muscle growth. The administration of exogenous pST to growing pigs has been shown to increase ADG by up to 30 %, increase muscle deposition rate by up to 50 %, and decrease fat deposition rate by up to 30 % (Etherton et al., 1986; Campbell et al., 1988; Dunshea et al., 2002), which prompted the development of an exogenous source of pST (Reporcin) that is approved for use in Australia. The current experiment was conducted to determine the effects of active immunization against GnRF combined with daily injections of pST on growth performance in group-housed boars and gilts.

MATERIALS AND METHODS

All procedures were approved by the Victorian Institute of Animal Science (VIAS) Animal Ethics Committee. Two hundred twenty four pigs (112 boars, 112 gilts; Large White x Landrace) were used in two replicates of a 2 x 2 x 2 factorial arrangement, with the factors being vaccination with Improvac, daily injection of pST, and gender. Pigs were selected for the experiment from the VIAS experimental herd at approximately 13 wk of age (8 wk preslaughter) and

blocked by sex, weight, and week of birth. At this time, pigs were randomly assigned to treatments and pigs selected to receive the Improvac (CSL Limited, Parkville, 3052 Australia) treatment were given their primary vaccination (2 mL/pig). Improvac is a vaccine against the animal's own GnRH. The control of testes function is regulated by GnRH produced by the hypothalamus. Improvac is an analogue of GnRH conjugated to a carrier protein in an aqueous adjuvant system. Its mode of action is immunological. The analogue has no hormonal or chemical activity. Improvac stimulates the pig's immune system to produce specific antibodies against GnRH. These antibodies inhibit natural GnRH activity, temporarily inhibiting testes function. Pigs remained in the VIAS herd for an additional four wk until approximately 17 wk of age (four wk preslaughter), at which time pigs received the secondary vaccination of Improvac (2 mL/pig) and all pigs were weighed and moved to the experimental facility (d = 0; initiation of study). Pigs were randomly assigned to one of 16 pens according to treatment and sex (7 pigs per pen; 3.7m^2 dry area per pen), and daily injections of pST (5 mg/d Reporcin) were initiated on pigs selected to receive pST treatment. Pigs were allowed to consume a standard wheat/soybean meal diet *ad libitum* (Table 1). Pigs were weighed and feed disappearance was measured weekly from the initiation of the experiment (17 wk of age) and continued until pigs were 21 wk of age, at which time pigs were sent to the abattoir for slaughter.

The median two pigs from each pen were chosen as focus pigs to determine changes in body composition in response to the various treatment combinations. Body composition was measured by dual-energy X-ray

absorptiometry (DXA) prior to the initiation of the experiment and then again prior to slaughter. Briefly, pigs were anesthetized by a ketamine/xylazine cocktail (10.0 and 1.0 mg/kg body weight, respectively) and were maintained under anesthesia with isoflurane while being scanned on the DXA. Regression equations were generated by Suster et al. (2000) on the same genotype and equipment as used for predictions of body composition in the present study.

Pigs were fasted overnight before being humanely slaughtered via CO_2 stunning at a commercial abattoir. The testes and ovaries were collected, trimmed, and weighed. Ovary scores were given on the basis of maturity (1 = immature, no follicles; 2 = small follicles; 3 = mature, very large follicles; 4 = mature, ruptured follicles).

Treatment effects were assessed by analyses of variance for a factorial design with the main effects being sex, Improvac vaccination and pST treatment. The effect of sex was not examined for testes weight (boars only) or ovary weight and score (gilts only). The experimental unit for all measures was the pen of pigs except for the body composition analyses where the experimental unit was the average of the 2 focus pigs per pen. For some measures (carcass weight, final tissue masses) initial live weight was used as a covariate to account for the small, non-significant differences in weights at the start of the study. All analyses were performed using GENSTAT (Payne *et al*. 1993).

RESULTS

Animal Performance

The initial stratification and randomization ensured that there was very little variation between average starting weights of treatment groups (within each sex group; Table 2). Surprisingly, there was no difference in live weight between boars and gilts at 17 wk of age (63.6 *v.* 64.7 kg for gilts and boars, respectively, $P = .70$). Over the first two wk of the study, ADG was lower for gilts than boars (1158 *v.* 1319 g/d, $P < .001$), but was unchanged by pST (1246 *v.* 1232 g/d, $P = .71$) and Improvac treatment (1210 *v.* 1267 g/d, $P = .16$). Over the ensuing two-wk period, ADG was lower for gilts than boars (1098 *v.* 1278 g/d, $P < .001$), and was increased by pST (1098 *v.* 1278 g/d, $P < .001$) and Improvac treatment (1090 *v.* 1286 g/d, $P < .001$). Over the entire 28 d of the study, ADG was lower for gilts than boars (1128 *v.* 1299 g/d, $P < .001$) and was increased by pST (1172 *v.* 1255 g/d, $P = .003$) and Improvac (1150 *v.* 1276 g/d, $P < .001$) treatments. Pigs, particularly boars, that received both pST and Improvac gained at a faster rate than pigs that received pST or Improvac alone.

Over the first two wk of the study, ADFI was lower for gilts than boars (2634 *v.* 2791 g/d, $P = .021$), was decreased by pST treatment (2819 *v.* 2606 g/d, $P = .003$) and increased by Improvac treatment (2626 *v.* 2799 g/d, $P = .012$; Table 2). However, there was a three-way interaction ($P = .012$) such that Improvac treatment increased ADFI in all classes of pigs except for untreated control boars. Over the ensuing two wk period, ADFI was lower for gilts than boars (2914 *v.* 3275 g/d, $P = .007$), was decreased by pST (3254 *v.* 2934 g/d, *P*

= .015) and was increased by Improvac treatment (2778 *v.* 3410 g/d, *P* < .001). When averaged over the entire 28 d of the study, ADFI was lower in gilts than boars (2774 *v.* 3033 g/d, *P* = .002), was decreased by pST (3037 *v.* 2770 g/d, *P* = .002) and increased by Improvac treatment (2702 *v.* 3105 g/d, *P* < .001). Pigs that received both pST and Improvac consumed feed at a similar rate as untreated control pigs.

Over the first two wk of the study feed conversion efficiency (FCE; gain:feed) was lower for gilts than for boars (0.437 *v.* 0.472, *P* = .011), was improved by pST treatment (0.439 *v.* 0.469, *P* = .017) but was unchanged by Improvac treatment (0.457 *v.* 0.450, *P* = .63; Table 2). Over the ensuing two wk period, FCE was not different between boars and gilts (0.370 *v.* 0.389, *P* = .21), was improved by pST (0.337 *v.* 0.435, *P* < .001) and unchanged by Improvac treatment (0.385 *v.* 0.373, *P* = .40). When averaged over the entire 28 d of the study, FCE was lower for gilts than for boars (0.403 *v.* 0.427, *P* = .025), was improved by pST (0.385 *v.* 0.452, *P* < .001) but unchanged by Improvac treatment (0.423 *v.* 0.410, *P* = .22). However, there was an interaction (*P* = .075) between sex and pST treatment such that FCE was lower in control gilts than in boars (0.366 *v.* 0.403) but not different between pST-treated gilts and boars (0.448 *v.*0.457). Pigs that received both pST and Improvac had a similar FCE as pigs treated with pST, both of which were higher than untreated controls.

Carcass Characteristics

At the end of the study at 21 wk of age, back fat depth at the P2 position was lower in gilts than in boars (13.6 *v.* 15.2 mm, *P* = .007), was decreased by

pST treatment (15.4 *v.* 13.4 mm, *P* = .001) and tended to be increased by Improvac treatment (13.9 *v.* 14.9 mm, *P* = .066; Table 2). There was also an interaction (*P* = .099) such that Improvac increased backfat depth in pigs not treated with pST (14.4 *v.* 16.3 mm), but not in pigs treated with pST (13.4 *v.* 13.5 mm). Carcass weight was lower in gilts than in boars (75.3 *v.* 77.0 kg, *P* = .012), was unchanged by pST treatment (75.9 *v.* 76.4 kg, *P* = .40) and increased by Improvac treatment (75.1 *v.* 77.2 kg, *P* = .003; Table 2). Pigs that received both pST and Improvac had similar carcass weights as pigs treated with Improvac, both of which were heavier than untreated controls. Dressing percentage was higher in gilts than in boars (78.5 *v.* 76.6 %, *P* = .006) but relatively unchanged by pST (78.0 *v.* 77.0 %, *P* = .12) and Improvac treatment (78.0 *v.* 77.1 %, *P* = 0.18), as well as pST and Improvac in combination (Table 2).

Body Composition and Tissue Accretion Rates

The allocation of the median two pigs as the focus pigs within each pen ensured that the focus pigs were essentially the same live weight as the entire pen of pigs they were chosen to represent (Table 3). In this respect, the average live weights were the same for the group and focus pigs (64.2 v. 64.3 kg, respectively, *P* = .72) and were highly correlated (group live weight = 2.24 (SE 3.65) + 0.967 (SE 0.0566) focus live weight, R^2 = .90, *P* < .001). Growth performance of the focus pigs was very similar to that of the groups of pigs they were chosen to represent with similar treatment effects to that observed for the analyses of the entire groups. At the start of the study, when the pigs were 17 wk of age and weighed approximately 64 kg, gilts contained more total body fat than boars (13.1 *v.* 11.2 kg,

P < .001) although there was no difference in lean (41.7 *v.* 42.0 kg, *P* = .56) or ash (1.51 *v.* 1.47 kg, *P* = .24) mass. As a percentage of live weight, gilts initially contained more fat (21.5 *v.* 18.7 %, *P* < .001), less lean tissue (68.7 *v.* 70.4 %, *P* < .001) but similar ash content (2.49 v 2.47 %, *P* = .65) to boars. Over the course of the study lean tissue deposition rate was lower in gilts than in boars (579 *v.* 725 g/d, *P* < .001), increased by pST (609 *v.* 696 g/d, *P* < .001) and by Improvac treatment (623 *v.* 682 g/d, *P* = .014; Table 3). However, there was a significant interaction (*P* = .031) in that pST increased lean tissue deposition rate to a greater extent in gilts than in boars. However, when combined with Improvac a greater response to pST was observed in boars compared to gilts, as indicated by a three-way interaction (*P* = .081). Pigs that received both pST and Improvac gained lean tissue at a faster rate than pigs that received pST or Improvac alone. This is particularly evident for boars, in that pST or Improvac treatment alone had no effect, while treatment with both pST and Improvac increased lean deposition rate by 16 %. Fat deposition rate tended to be lower in gilts than in boars (214 *v.* 247 g/d, *P* = .063), decreased by pST treatment (263 *v.* 198 g/d, *P* < .001) and increased by Improvac treatment (197 *v.* 264 g/d, *P* < .001). Pigs that received both pST and Improvac gained fat tissue at a similar rate as untreated controls. Ash deposition rate was lower in gilts than in boars (21.6 *v.* 26.9 g/d, *P* = .024) but not altered by pST (23.7 *v.* 24.9 g/d, *P* < .60) or Improvac treatment (23.7 *v.* 24.8 g/d, *P* = .61). Largely as a result of the differing rates of tissue deposition during the experiment, gilts contained less lean tissue (57.9 *v.* 62.4 kg, *P* < .001) but similar fat (19.2 *v.* 18.2 kg, *P* = .22) and ash (2.13 *v.* 2.22 kg, *P* =.23) in their body at slaughter compared to boars. Porcine

somatotropin treatment increased the lean tissue (58.9 *v.* 61.4 kg, *P* = .002), decreased the fat (19.7 *v.* 17.7 kg, *P* = .018) but had no effect on the ash (2.17 *v.* 2.17 kg, *P* = .99) mass in the body at slaughter. Improvac had no significant effect on the lean (59.8 *v.* 60.5 kg, *P* = .40) or ash (2.16 *v.* 2.18 kg, *P* = 0.83) but increased fat (17.8 *v.* 19.6 kg, *P* = .025) mass in the body at slaughter.

Sexual Characteristics

Testes weight at slaughter was decreased by Improvac treatment (354 *v.* 181 g, *P* < .001) but was not altered by pST treatment (256 *v.* 279 g, *P* = .44; Table 4). Ovary weight at slaughter was decreased by Improvac treatment (6.99 *v.* 2.99 g, *P* < .001) but was not altered by pST treatment (4.77 *v.* 5.21 g, *P* = .52). Ovary maturity score was decreased by Improvac treatment (2.4 *v.* 1.3, *P* < .001) but not altered by pST treatment (1.9 *v.* 1.8, *P* = .63).

DISCUSSION

The overwhelming finding from the present study was that the effects of sex, pST and Improvac on growth performance are additive or synergistic in group-housed finisher boars and gilts and that these technologies can be used in combination to dramatically improve growth performance and carcass composition. Thus, boars treated with both Improvac and pST had daily gains that were 43 and 64 % greater than untreated control gilts over the entire four or last two wk of the treatment period, respectively. Importantly, the effects of the combined treatment with pST and Improvac were demonstrated in gilts as well as in boars. These data are qualitatively similar to those observed in individually-

housed gilts and boars treated with both pST and Improvac (McCauley et al., 2002).

Porcine somatotropin treatment improved growth performance in both sexes, although responses were more apparent in the gilts. The improvements in performance of gilts treated with pST alone were quite remarkable with 17 and 29 % increases in daily gain and lean tissue deposition and 19 and 22 % improvements in FCE and fat deposition, respectively. While pST treatment did not markedly improve ADG (+3 %) or lean tissue deposition (-4 %), it did increase FCE (+13 %) and reduce fat deposition (-27 %) in boars. Others have also reported that pST treatment reduced or negated the sex differences normally observed over the finishing phase (Campbell et al., 1989; Dunshea et al., 2002; McCauley et al., 2002). Thus, while benefits of pST are greatest in gilts, even boars with high lean growth potential still respond positively to pST, at least with respect to feed efficiency and body composition.

Improvac treatment did not appear to have a major effect on growth performance for the first two wk after the second vaccination, although feed intake was increased. However, over the second two-wk period, pigs treated with Improvac had a higher ADG and ADFI. Over the entire four-wk treatment period, Improvac alone increased feed intake and daily gain by 18 %, without altering FCE, compared to untreated control gilts. Similarly, Improvac increased feed intake and daily gain by 13 and 9 %, respectively compared to untreated control boars, without any effect on FCE (-4 %). In another study with group-housed boars, Improvac increased daily gain by 10 and 30 % and feed intake by

15 and 21 % without changing FCE during the last four wk in boars slaughtered at 23 and 26 wk, respectively (Dunshea et al., 2001). As with the present study, most of the performance responses were observed in the third and fourth wk after vaccination. In individually-housed gilts, Improvac increased daily gain and feed intake by 10 and 11 %, respectively, without changing FCE (McCauley et al., 2002). In contrast, Improvac had no effect on feed intake or daily gain in individually-housed boars (McCauley et al., 2002). Improvac has been shown to reduce socio-sexual behavior to levels normally observed in surgical castrates (Cronin et al., 2001). The failure to observe a growth response due to Improvac treatment in the study by McCauley et al. (2002) may be due to the limited sexual and aggressive behavior of individually-housed boars. Similarly, McCauley et al. (2000) found that Improvac increased daily gain more in group housed (18 %) than individually-housed (11 %) boars. Therefore, there is now a body of evidence that vaccination against GnRF improves growth performance in group-housed boars and gilts at least in part through reducing sexual and aggressive behaviors.

Housing conditions and the quality of the environment impacts animal performance and the response to metabolic modifiers (NRC, 1994). Group-housed boars have lower growth rates and ADFI than individually-housed boars, presumably due to socio-aggressive behaviors (Black et al., 2001). In the present study, group-housed boars that received pST treatment alone did not exhibit increased lean tissue deposition. However, in individually-housed boars, pST increased lean tissue deposition by 16 % (McCauley et al., 2002),

presumably because of high feed intakes that resulted from the lack of adverse behaviors in group-housed animals. In situations of feed restriction, such as group-housed boars exhibiting sexual and aggressive behaviors, the further reduction in feed intake associated with pST may limit energy intake available for lean tissue deposition. Similar to pST, Improvac alone did not stimulate lean tissue deposition in the current study even though feed intake was increased, and thus the additional energy was deposited as fat. However, in the boars treated with both pST and Improvac, the additional energy was used for the anabolic effects of pST on lean tissue deposition. Improvac treatment seemed to ameliorate the feed restriction associated with group-housed boars by removing the adverse effects of sexual and aggressive behavior, thus allowing lean tissue deposition to increase. Improvac and pST had qualitatively similar effects in gilts, although the lower rate of lean tissue deposition in the untreated control gilts allowed increases in lean tissue deposition to be expressed in response to both technologies. In a companion study, conducted with individually-housed pigs, the additional energy consumed by pigs that received both Improvac and pST, compared to pST alone, was used for fat rather than lean tissue deposition (McCauley et al., 2002). However, the present study demonstrates that the combined treatments can be beneficial in stimulating lean tissue deposition under circumstances where energy intake is limiting lean tissue deposition such as in group-housed animals, especially boars. These effects may be even more pronounced when pigs are maintained at a high stocking density or under hot

conditions, or in genotypes exhibiting a low intrinsic propensity for fat deposition and feed consumption.

Improvac was initially developed to reduce the boar taint compounds, skatole and androstenone, in pork from boars to levels well below the detection threshold for consumers (Dunshea et al., 2001). In the present study, these compounds were not measured but the effects on testes and ovary weights, and ovary scores at slaughter, demonstrate the efficacy of the vaccine to suppress sexual development. Testes and ovary weights were reduced approximately 57 and 58 %, respectively by treatment with Improvac. In addition, ovaries were approximately 50 % less mature in Improvac treated gilts than those from control gilts. Dunshea et al. (2001) also observed decreased testes weight with a concomitant decrease in the odor causing compounds androstenone and skatole. Although not measured, we expect similar observations to have occurred in the present study. Therefore, the administration of Improvac had the desired effects on arresting sexual development in both boars and gilts in the current study. Similarly to McCauley et al. (2002), administration of pST had no effect on testes weight, ovary weight, or ovary score in the current study.

IMPLICATIONS

The results of the current experiment confirm earlier results that Improvac can arrest and reverse sexual development in boars. In addition, Improvac is efficacious in inhibiting sexual development in gilts. Furthermore, the concomitant use of Improvac and pST resulted in increased growth performance and lean tissue accretion above control levels and above pigs treated with

Improvac or pST alone, particularly in group-housed boars. The use of Improvac delays sexual maturity, thus reducing the occurrence of boar taint, and when used alone or with pST improves growth performance in group housed boars and gilts.

Table 1. Composition and calculated analysis of the diet[a]

Item	% of total
Wheat	79.74
Soybean Meal	6.00
Fish Meal	5.00
Blood Meal	2.50
Meat and Bone Meal	2.50
Tallow	1.77
L lysine•HCl	0.12
DL Methionine	0.005
L Threonine	0.019
Dicalcium Phosphate	1.48
Limestone	0.46
Salt	0.20
Vitamin and Mineral Premix	0.20
Calculated analysis	
DE, MJ/kg	14.50
Crude Protein, %	20.00
Lysine, g/MJ DE	0.65
Fat, %	4.31
Calcium, %	1.09
Phosphorus, available, %	0.60

[a]Expressed on an as fed basis

Table 2. Effect of sex, porcine somatotropin (pST) and Improvac on growth performance of finisher pigs[ab]

	Gilts				Boars				s.e.d.[c]	Significance[d]						
	CNTL	I	ST	I +ST	CNTL	I	ST	I +ST		S	ST	I	SxST	SxI	STxI	SxSTxI
Live weight (kg)																
d 0	64.4	65.4	63.5	61.2	64.7	64.9	66.2	63.0	5.6	.70	.63	.70	.68	.87	.55	1.00
d 14	79.5	82.6	79.7	77.4	83.8	83.3	83.7	81.7	5.8	.26	.57	.89	.78	.78	.57	.74
d 28	91.9	98.1	95.7	95.0	98.8	101.9	101.3	102.1	5.6	.046	.76	.41	.86	.90	.42	.69
Daily gain (g/d)																
d 0 to 14	1084	1226	1152	1170	1360	1314	1245	1359	77	<.001	.71	.16	.60	.56	.82	.081
d 14 to 28	885	1106	1146	1254	1070	1330	1258	1453	58	<.001	<.001	<.001	.40	.29	.14	.69
d 0 to 28	985	1166	1149	1212	1215	1322	1251	1406	49	<.001	.003	<.001	.37	.87	.49	.11
Feed intake (g/d)																
d 0 to 14	2630	2934	2465	2507	2870	2842	2538	2913	126	.021	.003	.012	.20	1.00	.58	.015
d 14 to 28	2751	3407	2604	2894	3019	3841	2741	3499	244	.007	.015	<.001	.93	.21	.39	.54
d 0 to 28	2690	3171	2535	2700	2945	3341	2639	3206	151	.002	.002	<.001	.54	.30	.64	.12
Feed conversion efficiency (Gain:Feed)																
d 0 to 14	0.41	0.42	0.46	0.47	0.47	0.46	0.49	0.47	0.12	.01	.017	.63	.10	.44	.64	.90
d 14 to 28	0.32	0.33	0.41	0.43	0.35	0.35	0.46	0.41	0.20	.21	<.001	.40	.23	.50	.49	.79
d 0 to 28	0.37	0.37	0.45	1.45	0.41	0.40	0.47	0.44	0.12	.03	<.001	.22	.075	.31	.57	.90
Carcass, kg[ef]	73.0	76.7	75.0	76.4	76.4	77.3	75.9	78.5	1.29	.012	.40	.003	.67	.58	.82	.14
Dressing %[d]	79.5	79.3	77.6	77.5	77.6	75.7	77.2	75.8	1.27	.006	.12	.18	.20	.28	.80	.89
P2 fat, mm[d]	14.2	15.6	12.5	12.2	14.7	17.0	14.3	14.8	1.04	.007	.001	.066	.26	.44	.099	.96

[a]Pigs were injected with pST (5 mg Reporcin®) daily from 17 until 21 wk of age (d 0 to 28 of treatment).

[b]Pigs received primary and secondary vaccination (2 mL Improvac®) at 13 and 17 wk of age, respectively. Pigs were slaughtered at 21 wk of age.

[c]Standard error of the difference of means for Sex x pST x Improvac.

[d]S = sex, ST = porcine somatotropin, I = Improvac.

[e]Determined at slaughter.

[f]Initial live weight used as a co-variate.

Table 3. Effect of sex, porcine somatotropin (pST) and Improvac on growth performance and tissue deposition of focus finisher pigs[abc]

	Gilts				Boars				s.e.d.[d]	Significance[e]						
	CNTL	I	ST	ST + I	CNTL	I	ST	ST + I		S	ST	I	SxST	SxI	STxI	SxSTxI
Live weight, kg																
d 0	65.2	65.7	64.4	62.4	64.7	63.9	64.0	64.1	5.77	.93	.69	.85	.76	.94	.89	.79
d 14	81.4	82.9	80.7	79.6	84.4	83.7	82.8	83.6	6.36	.45	.66	.97	.86	.98	.93	.76
d 28	94.8	97.4	95.1	95.3	97.8	102.6	97.9	103.4	6.20	.14	.94	.30	.83	.54	.90	.80
Daily gain, g/d																
d 0 to 14	1152	1228	1165	1228	1406	1411	1344	1393	114.9	.002	.77	.41	.69	.72	.89	.80
d 14 to 28	960	1036	1031	1121	960	1353	1076	1420	100.3	.003	.10	<.001	.90	.009	.86	.76
d 0 to 28	1056	1132	1098	1174	1183	1382	1210	1406	80.3	<.001	.41	.002	.84	.14	.99	.99
Final composition, kg[fg]																
Lean	55.9	56.3	59.7	59.8	61.7	61.5	62.0	64.3	1.49	<.001	.002	.40	.17	.61	.47	.35
Fat	19.6	20.9	17.0	19.2	17.6	20.5	16.9	17.8	1.51	.22	.018	.025	.77	.93	.74	.34
Ash	2.12	2.05	2.15	2.17	2.21	2.30	2.17	2.20	0.156	.23	1.00	.83	.36	.59	.93	.63
Final composition, %[g]																
Lean	67.3	66.7	69.6	68.3	69.5	67.8	70.1	70.0	1.19	.028	.009	.14	.69	1.00	.69	.33
Fat	23.5	24.5	19.9	22.2	20.0	22.7	19.1	19.4	1.18	.019	.009	.078	.65	.93	.78	.31
Ash	2.55	2.41	2.52	2.51	2.51	2.55	2.47	2.40	0.176	.88	.78	.62	.48	.76	.93	.50
Tissue deposition, g/d																
Lean	487	533	630	665	707	708	667	820	44.5	<.001	<.001	.014	.031	.42	.13	.08
Fat	211	270	164	210	238	333	174	243	34.0	.063	<.001	<.001	.50	.39	.57	.85
Ash	20.9	19.3	21.9	24.3	26.3	28.3	25.7	27.5	4.42	.024	.60	.61	.41	.74	.67	.64

[a]Pigs were injected with pST (5 mg Reporcin®) daily from 17 until 21 wk of age (d 0 to 28 of treatment).

[b]Pigs received primary and secondary vaccination (2 mL Improvac®) at 13 and 17 wk of age, respectively. Pigs were slaughtered at 21 wk of age.

[c]Data are based on the performance and body composition changes of 2 focus pigs per pen of pigs. Body composition determined by dual energy X-ray absorptiometry at the beginning and end of the study.

[d]Standard error of the difference of means for Sex x pST x Improvac.

[e]S = sex, ST = porcine somatotropin, I = Improvac.

[f]Initial live weight used as a co-variate.

[g]At the initiation of the experiment (d0), no differences in body composition were observed within each sex, regardless of treatment ($P > .36$). Fat and lean percentages were lower and higher, respectively, for boars compared to gilts ($P < .003$).

Table 4. Effect of sex, porcine somatotropin (pST) and Improvac on testes and ovary weights and ovarian maturity in finisher pigs[abc]

	Gilt				Boar				s.e.d.[d]	Significance[e]		
	Control	I	ST	ST + I	Control	I	ST	ST + I		ST	I	STxI
Testes (g)					356	156	352	207	41.1	.44	<.001	.36
Ovaries (g)	6.74	2.79	7.24	3.19					0.964	.52	<.001	.95
Ovary score[f]	2.50	1.26	2.33	1.26					0.246	.63	<.001	.63

[a]Pigs were injected with pST (5 mg Reporcin®) daily from 17 until 21 wk of age (d 0 to 28 of treatment).
[b]Pigs received primary and secondary vaccination (2 mL Improvac®) at 13 and 17 wk of age, respectively. Pigs were slaughtered at 21 wk of age.
[c]Determined at slaughter.
[d]Standard error of the difference of means for pST x Improvac.
[e]ST = porcine somatotropin, I = Improvac.
[f]Ovary scores are on a scale of 1 to 4 (1 = Immature, no follicles or pre-follicles; 2 =pre-follicles or small follicles visible; 3 = functioning ovaries, large follicles visible; 4 = large follicles present and ruptured follicles present).

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CHAPTER 3

PIGS WEANED FROM THE SOW AT 10 D OF AGE RESPOND TO DIETARY ENERGY SOURCE OF MANUFACTURED LIQUID DIETS AND EXOGENOUS PORCINE SOMATOTROPIN (pST)

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ABSTRACT

Previous research suggests that the neonatal pig does not alter feed intake in response to changes in the energy density of manufactured liquid diets. Also, the limited response of IGF-I to exogenous pST previously observed in young pigs may be influenced by the source of dietary energy. Our objectives were: 1) to determine the effect of a high (25% fat, ME=4639 kcal/kg; HF) or low (2% fat, ME=3481 kcal/kg; LF) fat manufactured liquid diet on pig performance, and 2) to determine if the limited response to exogenous pST in young pigs is dependent on the source of dietary energy. Two replicates of 60 pigs (n=120; barrows and gilts distributed evenly), with an initial body weight of 4207 ± 51 g, were weaned from the sow at 10 d of age and utilized in a randomized complete block design. Pigs were assigned by weight to 1 of 6 pens. Diets were formulated to provide a constant lysine:ME ratio and were fed on a pen basis for a duration of 9 d. On d 5, barrows and gilts within a pen were randomly assigned to receive either 0 or 120 μg pST•kg body weight $^{-1}$•day $^{-1}$ for 4 d. Pigs gained 335 ± 9 g/d, which resulted in an ending body weight of 7225 ± 95 g ($P > 0.42$). Pigs fed the LF diet consumed 17 % greater dry matter/pen/d than pigs fed the HF diet (2777 ± 67 vs 2376 ± 67 g/d, $P < 0.01$). Dietary treatment differences in ADFI were not observed on d 5 or 6 of treatment ($P > 0.25$). Calculated ME intake did not differ between dietary treatments ($P > 0.20$), except on d 5 and 6 of treatment, where ME intake was higher in the pigs fed the HF diet ($P < 0.05$). Feed conversion (gain:feed) was 24 % higher in HF compared to LF fed pigs ($P < 0.001$). Plasma urea nitrogen concentrations were higher in the HF fed

pigs (11.1 ± 0.6 mg/dL), compared to pigs fed the LF diet (6.4 ± 0.6 mg/dL, $P < 0.001$). Treatment with pST increased circulating IGF-I ($P < 0.001$) and decreased PUN ($P < 0.003$) concentration 32 and 25 %, respectively, regardless of dietary treatment ($P > 0.65$). Circulating leptin averaged 1.8 ± 0.1 ng/mL and was not affected by dietary treatment ($P > 0.26$) or pST ($P > 0.54$). These results suggest that the ST/IGF axis is responsive to exogenous pST in the young pig and the increase in circulating IGF-I and growth is independent of the source of dietary energy. Also, young pigs respond to a lower energy density liquid diet with increased feed intake, without altering growth performance, apparently utilizing a mechanism other than circulating leptin.

Key Words: swine, energy source, somatotropin, IGF-I, leptin

INTRODUCTION

Improvements through selection, management practices, and nutrition have resulted in dramatic increases in postweaning growth performance of swine, but growth rates have not been improved during the nursing phase of production. Increased weaning weight reduces postweaning mortality and growth lag, improves nursery performance, and ultimately decreases the age at market weight (Harrell et al., 1993; Kim et al., 2001). Supplemental feeding strategies of nursing pigs provide evidence that the lactating sow does not optimize baby pig growth (Kelly et al., 1990; Azain et al., 1996). In addition, results from artificial rearing studies have indicated that sow's milk does not supply a pattern of nutrients for maximal piglet growth rate

(Auldist et al., 1997). For example, twenty-one day weights of over 9.5 kg are caused by feeding manufactured liquid diets (Harrell et al., 1993; Oliver et al., 2002). Also, fat provides approximately 50 % of the total calories in sow's milk (Klobasa et al., 1987), which suggests that young pigs require a high amount of dietary fat.

Porcine somatotropin has been utilized effectively to alter the partitioning of nutrients away from fat and towards muscle growth. The administration of exogenous pST to growing pigs has increased ADG by up to 30 %, increased muscle deposition rate by up to 50 %, and decreased fat deposition rate by up to 30 % (Etherton et al., 1986; Campbell et al., 1988; Dunshea et al., 2002). The response to pST is dependent on adequate nutrient intake (Campbell et al., 1991; Krick et al., 1993) and stage of development (Harrell et al., 1999). High dietary fat intake may limit the response to pST, as indicated by a greater IGF-I response to ST in humans on a high, compared to a low carbohydrate diet (Snyder et al., 1989). Therefore, this experiment was conducted to determine if young pigs alter feed intake in response to dietary energy source (lipid vs carbohydrate), and if dietary energy source affects the response to pST.

MATERIALS AND METHODS

Animal Care and Dietary Treatments. All procedures were approved by the Institutional Animal Care and Use Committee of North Carolina State University. Two replicates of 60 pigs (n=120; barrows and gilts distributed evenly) were weaned from the sow at 10 d of age and utilized in a randomized complete block design.

Pigs were blocked by weight and assigned to 1 of 6 pens (10 pigs/pen). Pigs were housed in a specialized nursery building (Intensive Care Nurseries, INC. Colfax, IL) with raised pens, with half of each pen containing an enclosed hover maintained at approximately 32°C, as described by Heo et al. (1999). Ambient temperature was maintained at approximately 24°C. Each block was randomly assigned to either a high (25%, HF) or low (2%, LF) fat diet. Diets were reconstituted at 150 g/L of water (approximately 12% dry matter) and were formulated such that the supply of amino acids per unit of energy was constant (Table 1). Manufactured liquid diet was delivered via a gravity flow feeding system similar to Oliver et al. (2002), with the exception of 30 L Nalgene Carboys (Fisher Scientific, Pittsburgh, PA) utilized to accommodate a pen of 10 pigs. Fresh manufactured liquid diet was added twice daily (0800 and 2000) for nine days to ensure freshness and *ad libitum* access to diets. Liquid diet was prepared on a daily basis and stored at 4° C. Feed disappearance and growth were measured gravimetrically on a daily basis. All components of the feeding system were cleaned daily prior to the first feeding (0800) with a liquid chlorinated detergent (DS Liquid: Command, Diversey Corp., Wyandotte MI).

Blood Collection and Analyses. On d 5, pigs (barrows and gilts) within a pen were randomly assigned to receive either 0 or 120 μg pST•kg body weight $^{-1}$•day $^{-1}$ (Reporcin solubilized in sterile water; Alpharma Co., Toorak, Australia) for 4 d in the extensor muscles of the neck. Pigs selected to receive the 0 pST treatment were injected with an equal volume of sterile saline. The dose of pST or volume of saline

was adjusted daily according to daily pig weights. Blood samples were collected via jugular venipuncture approximately 18 h after the final injection of exogenous pST and immediately placed on ice. After collection, blood samples were centrifuged (Beckman, model J-B6) at 824 x *g* for 10 min with plasma collected and frozen at – 20° C until further analysis. Plasma was analyzed in duplicate for urea nitrogen, IGF-I, NEFA, and leptin concentrations. Plasma urea nitrogen (PUN) concentrations were determined by the quantitative urease/Berthelot procedure (Sigma Diagnostics, St. Louis, MO) based on methods described by Fawcett and Scott (1960), and Chaney and Marback (1962). Plasma NEFA concentrations were determined by an enzymatic colorimetric method (Wako Pure Chimical Industries, Ltd., Richmond, VA). The sample mean for PUN pools was 6.75 ± 0.07 mg/dL and the intraassay CV was 2.0 %. The sample mean for NEFA pools was 169.6 ± 2.3 mEq/L and the intraassay CV was 2.7 %.

Plasma IGF-I and leptin concentrations were analyzed by radio-immuno assays. Plasma IGF-I was dissociated from the IGF binding proteins (IGFBP) using the glycyl-glycine extraction method as described by Plaut et al. (1991). For the analysis of IGF-I, recombinant human IGF-I was used for standards (Gropep, Ltd., Adelaide, Australia), as well as the radiolabeled ligand (Amersham Parmacia Biotech UK Ltd., Buckinghamshire, England). Anti-human IGF-I polyclonal antiserum (GroPep) was used as the primary antibody and goat anti-rabbit gamma globulin (GroPep) was used as the second antibody. The sample means for IGF-I pools were 222 ± 4 and 335 ± 18 ng/mL and the intraassay CVs were 3.4 and 10.7

%, respectively. For the analysis of leptin, recombinant human leptin was used for standards and radiolabeled ligand (Linco Research, Inc., St. Charles, MO). Guinea pig anti-multi-species leptin antibody was used as the primary antibody (Linco Research, Inc.). These reagents were validated for use in the measurement of leptin in porcine serum in our laboratory (Whisnant and Harrell, 2002). The sample mean for the leptin pool was 3.48 ± 0.04 ng/mL and the inter- and intraassay CVs were 2.4 and 3.7 %, respectively.

Statistical Analysis. Data were subjected to analysis of variance using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). Data were evaluated for the effects of energy source, pST, replication, and all appropriate interactions. The energy source (fat vs. carbohydrate) and pST (0 vs 120 μg pST•kg body weight $^{-1}$•day $^{-1}$) response were contrasted using a protected least significant difference test (Steel et al., 1997). The experimental unit for all analyses was pen of pigs. The average value of all pigs within a pen was used for the evaluation of energy source effects and the average value of all pigs receiving either the 0 or 120 μg pST•kg body weight $^{-1}$•day $^{-1}$ within a pen was used for the evaluation of pST effects. The significance level for all tests was set at $P < .05$.

RESULTS

Pigs gained 335 ± 9 g/d, which resulted in an ending body weight of 7225 ± 95 g (Table 2), regardless of dietary treatment ($P > 0.42$). Pigs consuming the LF

diet had 17% greater (2777 ± 67 dry matter/pen/d, Figure 1) ADFI, compared to pigs consuming the HF diet (2376 ± 67 g dry matter/pen/d, $P < 0.01$). Treatment differences in ADFI were not observed on d 5 or 6 of treatment ($P > 0.25$). Estimated metabolizable energy (ME) intake did not differ (Figure 2, $P > 0.20$), except on d 5 and 6 of treatment where pigs fed the HF diet had higher ME intakes than pigs fed the LF diet ($P < 0.05$). As a result of the changes in feed intake and no changes in ADG, feed conversion (gain:feed) was increased by 24 % in pigs fed HF compared to LF diets (Table 2, $P < 0.001$). Pigs that received exogenous pST tended to gain weight faster than pigs that did not receive pST, regardless of dietary treatment (Table 3; $P < 0.10$).

Dietary treatment had no effect on basal circulating IGF-I concentrations (Table 3, $P > 0.49$). However, circulating IGF-I concentrations were increased ($P < 0.001$) by 32 % in pigs treated with pST, regardless of dietary treatment ($P > 0.78$). Plasma urea nitrogen concentrations were 42 % lower in the LF fed pigs, compared to the HF fed pigs (Table 3, $P < 0.001$). In addition, exogenous pST decreased PUN concentrations by 25 % in both LF and HF fed pigs ($P < 0.003$). Plasma NEFA concentrations were 56 % lower in pigs consuming the LF diet, compared to pigs fed the HF diet (Table 3, $P < 0.001$). Plasma NEFA concentrations were not affected by pST ($P > 0.40$). Circulating leptin concentrations (Table 3) averaged 1.8 ± 0.1 ng/mL and were not affected by dietary treatment ($P > 0.26$) or exogenous pST ($P > 0.54$).

DISCUSSION

The most convincing data that preweaning pig growth is not maximized by the lactating sow are results from artificial rearing studies (Harrell et al., 1993; Oliver et al., 2002). Harrell et al. (1993) found that artificially reared pigs were 53 % heavier at 21 d of age compared to pigs reared on the sow (9.8 vs. 6.4 ± 0.5 kg) and the increased preweaning growth resulted in 10 fewer d to reach 110 kg body weight. Similar 21-d weights were found by Oliver et al. (2002) who examined corn syrup solids as a replacement for lactose in manufactured liquid diets in pigs from 2 to 21 d of age. In the current experiment, overall ADG was 335 ± 8 g/d. This level of performance is consistent with previous research with manufactured liquid diets (Newport 1979; Heo et al., 1999), in that pigs in the current study performed superior to pigs typically reared on the sow (250 g/d; NRC, 1998). Increased growth of pigs reared independent of the sow could be accomplished with increased supply and(or) improved nutrient profile of manufactured liquid diet.

Extensive research has been conducted to optimize the supply of nutrients to maximize lean tissue gain during the post-weaning phases of growth, but little has been done in the nursing phases through either changes in sow milk composition or manufactured liquid diets. Fat provides approximately 50% of the total calories in sow's milk (Klobasa et al., 1987), which suggests that the young pig (i.e. < 18 d of age) requires a high amount of dietary fat in order to maximize their weight gains. However, pigs fed the HF or LF diets throughout the current study gained at similar rates (340 ± 9 and 330 ± 9 g/d, respectively), resulting in ending weights of 7270 ±

95 and 7180 ± 96 g, respectively. Similarly, Cline et al., (1977) observed no difference in growth rates of pigs weaned at three wks of age and limit fed isocaloric high or low fat liquid diets for two wks. These data indicate that young pigs are capable of using either fat or carbohydrate equally well as the primary energy source for growth.

In growing pigs, increased dietary energy concentration resulted in decreased feed intake, while metabolizable energy intake remains relatively constant (NRC, 1987). In addition, supplemental fat (10 %) decreased feed intake in nursery pigs (Cera et al., 1990; Li et al., 1990). However, Le Dividich et al. (1997) concluded that one-d old pigs did not respond to colostral energy concentration with increased feed intake. While pigs were not allowed *ad libitum* access to the diet, these results may still be accurate due to the limited gastric capacity of pigs at one-d of age. In the current experiment, 10-d old pigs that received the LF diet *ad libitum* consumed 17 % more feed, compared to HF fed pigs. Due to the differences in feed intake with no change in growth rate, feed conversion (gain:feed) was 24 % higher in HF compared to LF fed pigs.

Leptin is involved in the regulation of feed intake and energy balance in a variety of species including rodents and humans (Ahima and Flier 2000). Leptin is secreted by adipose tissue and travels via the circulation bound to a specific binding protein, which may modulate its activity, to the brain where it inhibits the release of the neurotransmitter, neuropeptide Y (Houseknecht et al., 1998). Neuropeptide Y is a potent stimulator of feed intake and inhibits thermogenesis. Therefore, the

changes in feed intake in response to energy density observed in the present study could be mediated through leptin. However, circulating leptin was unaffected by dietary energy source in the current experiment, despite differences in the level of feed intake and similar ME intakes. In contrast, high fat diets decreased circulating leptin in both rats (Ainslie et al., 2000) and humans (Romon et al., 1999). In addition, Cha et al. (2000) observed that high fat diets (44 or 60 %) abolished the diurnal variation of leptin normally observed in rats.

Circulating leptin also was unaffected by exogenous pST in pigs in the present study. However, in older pigs allowed to consume feed *ad libitum*, exogenous pST administration transiently increased circulating leptin concentrations by two h post treatment, and returned to baseline by six h post treatment (Harrell, unpublished data; Figure 3). Circulating levels of leptin were lower at 12, 24, 48, and 56 h after the initial pST injection, compared to baseline values. Also, no diurnal pattern was observed when pigs were bled at hourly intervals for 24 h (data not shown). Due to the transient increase of the initial circulating leptin response to exogenous pST, and inconsistent response after later injections (> 6 h), changes in leptin may not have been observed due to the time of blood sampling. Elimam et al. (2001) and Matsuoka et al. (1999) both observed decreased circulating leptin in children in response to ST administration. However, these children were either severely obese (Elimam et al., 2001) or had decreased somatotropin secretory capacities (Matsuoka et al., 1999). Matsuoka et al. (1999) observed that leptin concentrations and adipose mass decreased proportionally, but Elimam et al. (2001)

observed a disproportionately greater reduction in leptin compared to fat mass, indicating that somatotropin had effects that were independent of the changes in total body adipose mass. In addition, Spurlock et al. (1998) observed lower leptin mRNA abundance in pigs treated with a single 2-mg dose of pST. In contrast, Iglesias et al. (2002) observed increased leptin concentrations after two wks of somatotropin treatment in dialysis patients with already elevated levels of leptin due to chronic renal disease.

Plasma urea nitrogen concentration is an indirect measure of the extent of amino acid oxidation, and in the young growing animal that is actively accreting skeletal muscle it is a measure of the oxidation of dietary amino acids. Circulating NEFA is an indirect measure of lipolysis and(or) fatty acids available for uptake into tissues. In the current study, levels of lipolysis should be very low in pigs weaned from the sow at 10 d of age. Plasma urea nitrogen and NEFA were both higher in pigs consuming the HF diet, compared to pigs fed the LF diet. Plasma urea nitrogen concentrations were approximately 32 % lower in LF fed pigs, compared to HF fed pigs. Due to similar ME intakes between diets in the current experiment, pigs fed the HF and LF diets consumed similar amounts of CP because the diets were formulated to have a constant CP:ME. Therefore, these data suggest that pigs fed HF diets were oxidizing more dietary amino acids than LF fed pigs, and indicates that HF pigs were accruing less muscle and therefore more fat than pigs consuming the LF diet. Similarly, circulating NEFA concentrations were approximately 56 % lower in pigs fed the LF compared to the HF diet. This was expected because pigs

fed the HF diet would have more dietary fatty acids available for tissue uptake. In addition, Tikofsky et al. (2001) observed that bull calves fed a low fat (14.8 %) diet had higher empty body protein and lower empty body fat, compared to calves that consumed a similar amount of energy from a high fat (30.6 %) milk replacer. Although we did not measure effects on body composition, these data suggest the efficiency for amino acid use for protein accretion is higher in pigs consuming a LF diet compared to a HF diet.

The effects of pST on the neonatal pig have received relatively little attention in comparison to their more mature counterparts, and the results obtained in young pigs have been inconsistent. Harrell et al. (1999) removed pigs from the sow at 2 to 3 d of age and challenged pigs with 120 μg/kg BW of pST daily for 4 d starting on 10, 19, 33, 43, 63, 83, and 125 d of age. Differences in response to pST at 10 and 19 d of age were observed in PUN, IGF-I, IGFBP-2, and IGFBP-3, albeit to a lesser extent than heavier pigs. Similar to Harrell et al. (1999), the increase in IGF-I and decrease in PUN in the current study indicates that the somatotropin/IGF axis is active in the young pig. In addition to changes in metabolic signals, we observed a growth response, in that ADG was increased by approximately 11 % in pigs treated with exogenous pST. However, we did not observe a difference in circulating NEFA due to exogenous pST, as found in older pigs (Wray-Cahen et al., 1991; Dunshea et al., 1992). Wester et al. (1998) also observed changes in IGF-I, IGFBP-2, and IGFBP-3 in pigs weaned from the sow at 12 h to 1 d of age and fed manufactured liquid diets. In addition, body weights for pigs that received pST were heavier after

the 7-d experiment. However, the levels of pST administered in this study were 10 to 15 fold higher (1 mg/kg BW given in 3 equal injections per day), compared to the more common dose of 120 μg pST•kg body weight $^{-1}$•day $^{-1}$ which maximized lean tissue deposition in growing pigs from 30 to 90 kg BW (Krick et al., 1992). In contrast, no differences were observed by Dunshea et al. (1999) in growth performance or circulating concentrations of IGF-I, IGF-II, or IGFBP-3 when daily injections of pST (0 or 60 μg/kg BW) were given to nursing pigs from d 4 to 31 of lactation. These data probably differ from other reports due to the lower dose of pST that was administered, which coincides with the concentration of pST approved for commercial use in Australia. Furthermore, at growth rates typical of pigs reared on the sow, the restraint of growth leading from a restricting amount of nutrients available from the sow could cause an attenuated response to pST. For example, when the effects of exogenous pST were titrated by different protein levels (8.3 to 23.4 g/100 g feed as-fed) the expected effects on growth performance and protein deposition were observed with the four highest protein diets, but not the two lowest protein diets (Campbell et al., 1990). In addition, circulating concentrations of IGF-I and IGFBP-3 were decreased, and concentrations of IGFBP-2 were elevated in conditions of poor nutritional status (Clemmons and Underwood 1991; Thissen et al., 1994; Underwood et al., 1994), which indicated a decreased responsiveness of the somatotropin/IGF axis. In contrast to Dunshea et al. (1999), Harrell et al. (1999), as well as the current experiment, observed that the somatotropin/IGF axis is functional

in pigs consuming manufactured liquid diets *ad libitum* growing at rates superior to pigs reared on the sow.

In the current experiment, the response to pST was similar between diets, indicating that the source of dietary energy does not affect the response to exogenous pST. In contrast, Snyder et al. (1989) observed lower urinary nitrogen losses and a greater IGF-I response to exogenous somatotropin (0.1 mg/kg initial BW every other day) in human subjects consuming 72 % of their non-protein calories as carbohydrate, compared to subjects consuming 80 % of their non-protein calories as lipid. However, these subjects were in a negative energy balance. Similar to the current experiment, Azain et al. (1992) observed no differences in response to pST in pigs consuming a diet with 10 % supplemental fat, compared to a diet with no supplemental fat, although these were older pigs (87 kg). These data, along with the current experiment, suggest that the responsiveness of the ST/IGF axis is not affected by dietary energy source.

This study confirms earlier data that the nutrient pattern of sow's milk may not be optimal for maximizing pig growth. For example, growth performance of pigs was maximized when the supply of lysine per unit of energy was approximately 50% higher than found in sow's milk (Auldist et al., 1997). Furthermore, diets that were utilized in artificial rearing studies, including the current study, supplied approximately 50% greater amino acid content per unit of energy than sow's milk and resulted in heavier pig weight gains (Harrell et al., 1993; Oliver et al., 2002). Also, supplementing lactating sow diets with conjugated linoleic acid reduced sow

milk fat content by approximately 35%, but growth performance of the nursing litters was not altered (Harrell et al., 2000). This study suggests that the somatotropin/IGF axis is active in the young pig, but is not influenced by the source of dietary energy.

IMPLICATIONS

The results from this experiment clearly show that the young pig (10 d of age) responds to the energy density of the diet. Pigs that received LF compared to HF diets had increased feed intake and similar ME intake, but resulted in similar rates of growth. In addition, the somatotropin/IGF axis appears to be responsive in the young pig and the increase in IGF-I and growth to exogenous pST is independent of the source of dietary energy. The differences in feed intake to obtain similar levels of ME intake utilize a different mechanism than circulating leptin. These data suggest that feed manufacturers could alter dietary formulations for early-weaned pigs fed manufactured liquid diets, depending on the availability and economics of dietary ingredients.

Table 1. Composition and calculated analysis of the dietary treatments[a]

Item	Diet	
	High Fat	Low Fat
Ingredients, %		
Non-fat dry milk[b]	45.85	42.00
High fat source[c]	30.10	1.50
Lactose[d]	0.00	39.70
Whey protein concentrate[e]	6.00	4.00
Na caseinate[f]	10.00	6.00
Arginine	0.30	0.22
L-Lysine•HCl	0.20	0.13
Xanthan gum	1.00	1.00
CaCO3	0.53	0.37
Dicalcium phosphate	3.75	2.81
Vitamin premix[g]	0.13	0.13
Mineral premix[h]	0.50	0.50
NaCl	0.88	0.88
$MgSO_4$	0.20	0.20
KCl	0.56	0.56
Calculated analysis[i]		
ME, kcal/kg	4639	3481
CP, %	31.15	23.50
Fat, %	24.97	1.89
Lactose, %	25.78	60.61
Lysine, %	2.74	2.05
Ca, %	1.11	0.82
P, %	0.76	0.56
g CP/Mcal ME	67.1	67.5
g Lysine/Mcal ME	5.9	5.9
g Lysine/100 g CP	8.8	8.8
g Ca/Mcal ME	2.4	2.4
g P/Mcal ME	1.6	1.6
Ca/P	1.5	1.45

[a]Expressed on a dry weight basis.

[b] Milk Specialties Corp. (Dundee, IL 60118).

[c] A blend of edible lard and fancy tallow (80% fat,; Fat Pak 80, Milk Specialties Corp., Dundee, IL 60118) containing 0.09% 12:0, 1.24% 14:0, 0.16% 15:0, 20.36% 16:0, 2.55% 16:1, 0.41% 17:0, 0.25% 17:1, 10.10% 18:0, 37.01% 18:1, 8.12% 18:2, 0.08% 18:3, 0.17% 20:0, 1.07% 20:1, 0.08% 20:2, 0.33% 20:4.

[d]Carl S. Akey, Inc. (Lewisburg, OH 45338).

[e]Whey Protein Concentrate (AMP 80, American Meat Protein Corp., Ames, IA 50010).

[f]International Ingredient Co. (St. Louis, MO, 63116).

[g]Vitamin premix (Milk Specialties Corp., Dundee, IL 60118) contained 33,000,000 IU/kg Vitamin A, 6,600,000 IU/kg Vitamin D_3, 55,000 IU/kg Vitamin E, 257,400 ppm Vitamin C, 29,983 ppm D-pantothenic acid, 33,069 ppm niacin, 8378 mg/kg riboflavin, 5,115 mg/kg menadione, 66 ppm biotin, 44,000 ppm Vitamin B_{12}, 2,038 ppm thiamine, 3,996 ppm Vitamin B_6, 2,756 ppm folic acid.

[h]Mineral premix (Milk Specialties Corp., Dundee, IL 60118) contained 1.002% Ca, 0.549% P, 0.284% Na, 0.040% Cl, 2.024% K, 0.102% Mg, 20,000 ppm Fe, 200 ppm Co, 1,850 ppm Cu, 400 ppm I, 5,000 ppm Mn, 60 ppm Se, 23,500 ppm Zn.

[i]Calculated analysis based on analysis provided by companies furnishing product and standard feed tables (NRC, 1998).

Table 2. Performance of young pigs fed a high (25 %) or low (2 %) fat manufactured liquid diet from d 10 to 19 of age.[a]

Item	Diet		SEM[c]	Significance[b]
	High Fat	Low Fat		ES
Live Weight, g				
d 10	4210	4204	51	>0.93
d 15	5656	5646	80	>0.93
d 19	7270	7180	95	>0.42
ADG, g/d				
d 10 to 15	289	291	8	>0.91
d 15 to 19	403	370	11	>0.22
d 10 to 19	340	330	9	>0.45
Gain/Feed				
d 10 to 15	1.53	1.29	0.02	<0.001
d 15 to 19	1.36	1.08	0.03	<0.001
d 10 to 19	1.44	1.16	0.02	<0.001

[a]Values are least squares means; n = 6.
[b]ES = energy source (fat vs carbohydrate).
[c]Standard error of the difference of the means

Table 3. Effects of exogenous porcine somatotropin (pST) and dietary energy source on ADG and plasma parameters of pigs fed a high (25%) or low (2%) fat manufactured liquid diet from d 10 to 19 of age.[a b]

Item	Diet				SEM[c]	Significance		
	High Fat	High Fat (pST)	Low Fat	Low Fat (pST)		ES[d]	pST	ESxpST
ADG, g/d								
d 15 to 19	388	418	353	402	21	>0.24	<0.10	>0.66
PUN, mg/dL	11.1	8.6	6.4	4.5	0.7	<0.001	<0.003	>0.65
IGF-I, ng/mL	278	360	258	350	22	>0.49	<0.001	>0.78
NEFA, μEq/L	191	192	84	43	23	<0.001	>0.40	>0.37
Leptin, ng/mL	1.8	1.7	1.9	1.8	0.1	>0.26	>0.54	>0.93

[a]Values are least squares means; n =6.

[b]Daily injections of 0 or 120 μg pST•kg body weight $^{-1}$•day $^{-1}$ for 4 d began on d 15 of age. Blood was collected approximately 18 h after the last pST injection.

[c]Standard error of the difference of the means.

[d]ES = energy source (fat vs carbohydrate).

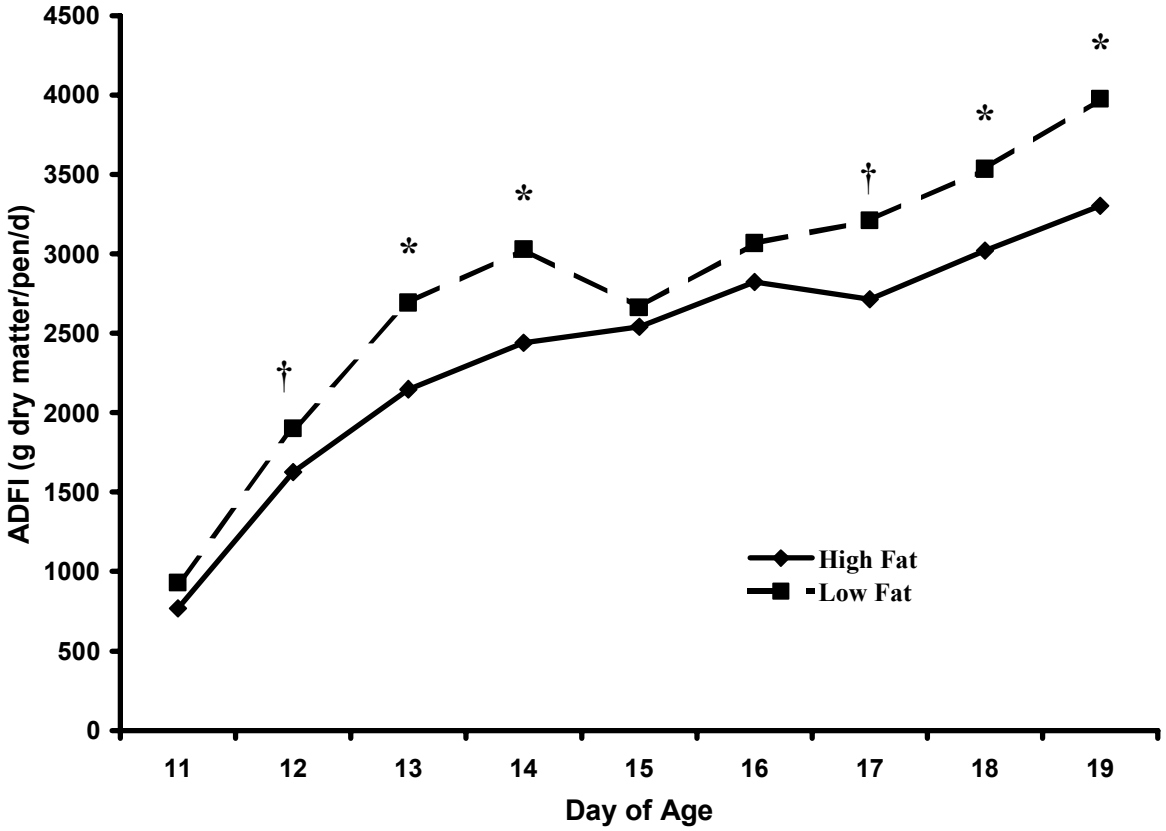

Figure 1. Average daily feed intake of pigs fed a high (25%) or low (2%) fat manufactured liquid diet from d 10 to 19 of age. Values shown are least squares means: n = 6. Pooled SEM = 87 †Diet effect ($P < 0.10$). *Diet effect ($P < 0.05$).

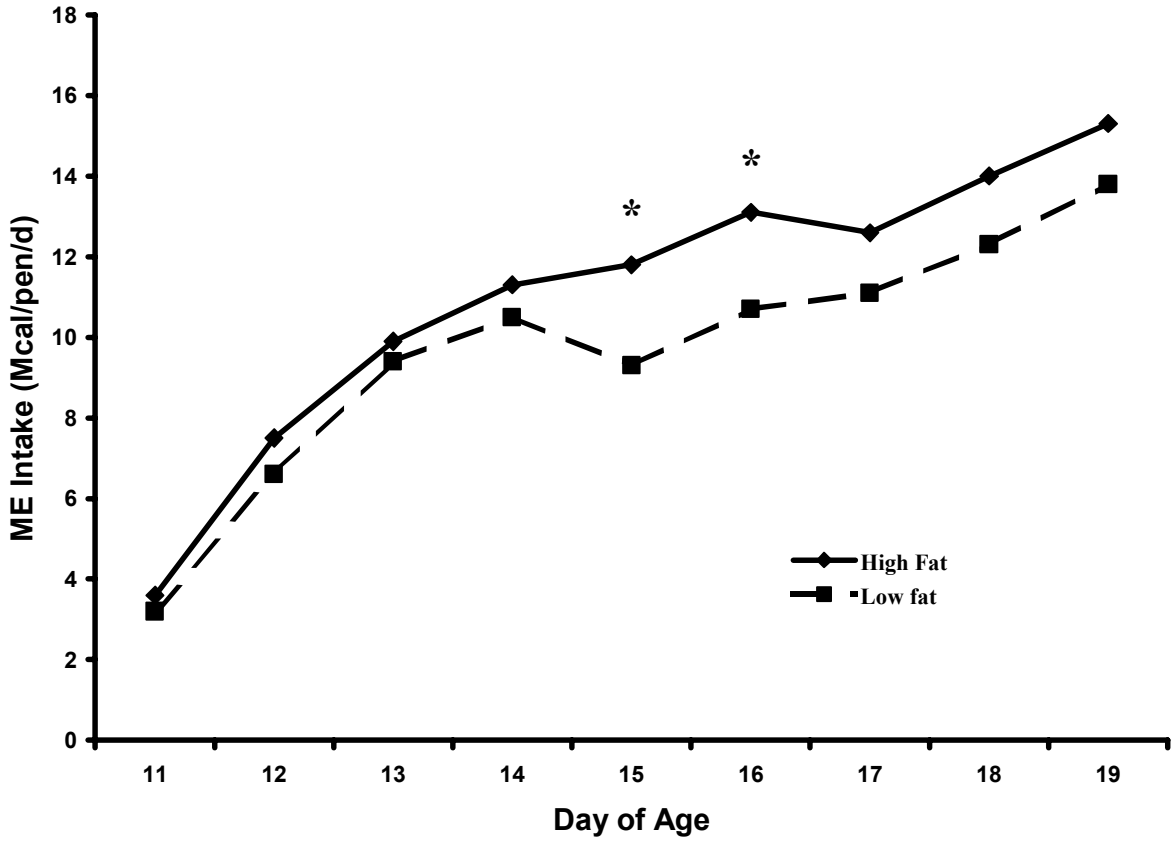

Figure 2. Daily estimated metabolizable energy (ME) intake of pigs fed a high (25%) or low (2%) fat manufactured liquid diet from d 10 to 19 of age. Values shown are least squares means: n = 6. Pooled SEM = 0.25 *Diet effect ($P <$ 0.05).

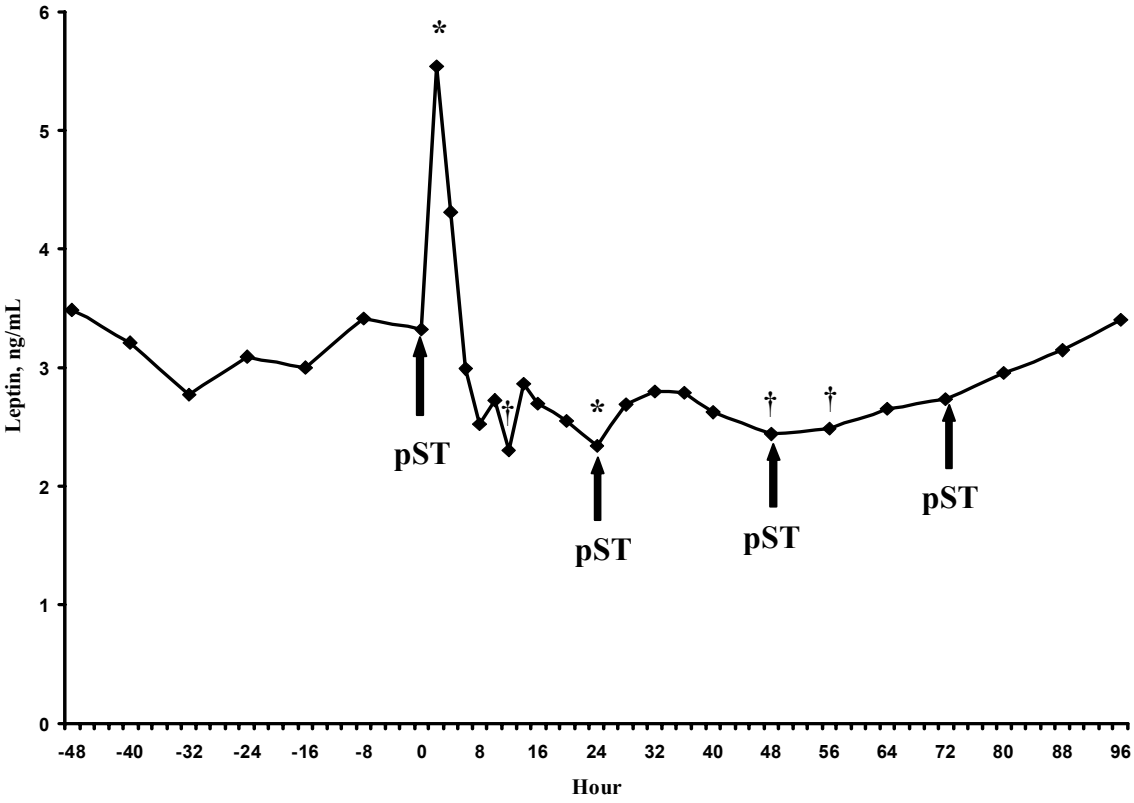

Figure 3. Effect of porcine somatotropin injection (pST) on circulating leptin concentrations in pigs weighing 82.0 ± 3.5 kg and allowed to consume feed *ad libitum*. Pigs (n = 8) were surgically fitted with indwelling jugular catheters and received 120 μg pST•kg body weight $^{-1}$•day $^{-1}$ for 4 d after a one week adjustment period. Blood samples were collected every eight h for 48 h before pST injections to establish baseline values. Blood samples were collected every two h for 16 h beginning immediately after the first pST injection. Blood samples were then taken every four h from 16 to 40 h after pST treatment and every eight hours from 40 to 96 h after pST treatment. Values shown are means. Pooled SEM = 0.11. †pST effect ($P < 0.10$). *pST effect ($P < 0.05$).

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CHAPTER 4

THE SOMATOTROPIN (ST)/INSULIN-LIKE GROWTH FACTOR (IGF) SYSTEM IS NOT AFFECTED BY AN INFECTIOUS DISEASE CHALLENGE IN GROWING PIGS

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ABSTRACT

Respiratory diseases account for considerable economic loss in the swine industry due, in part, to reduced growth performance. The objectives of this experiment were: 1) to determine the effect of porcine reproductive and respiratory syndrome virus (PRRSv) and *M. hyopneumoniae* (*M. hyo.*) on basal IGF-I concentrations, and 2) to determine if exogenous ST can stimulate the IGF system in health-challenged pigs. Pigs were randomly assigned to one of three treatment groups: 1) Non-infected, *ad libitum* intake (C); 2) Challenged with PRRSv and *M. hyo.*, *ad libitum* intake (HC); and 3) Non-infected, pair-fed to HC pigs (PF). HC pigs were infected with *M. hyo.* 8 days prior to PRRSv infection (d 0). On d 14 of infection, pigs within a treatment group were randomly assigned to receive either 0 or 120 μg porcine ST•kg body weight $^{-1}$•day $^{-1}$ for 4 days. Only infected pigs had lung lesions typical of *M. hyo.* on d 21. Initial body weight did not differ (P>0.64), but C pigs were heavier than both the HC and PF groups on d 21 (P<0.01). From d 0 to 21, C pigs gained at a faster rate than HC and PF pigs (P<0.01). Disease challenge reduced feed intake beginning on d 7 of infection (P<0.04), with a maximum reduction of ~50 % (P<0.01) on d 16. Disease challenge did not affect feed conversion (G:F, P>0.10), but PF pigs had a 17 % lower G:F than HC pigs (P<0.02). Basal circulating IGF-I levels were 322±22 ng/mL, regardless of treatment (P>0.15). ST administration increased circulating IGF-I 2.5-fold (P<0.01), regardless of treatment. On d 14 after PRRSv infection, the C pigs had a higher plasma insulin concentration, compared to NC pigs (P < 0.02), but were not different on x days. Insulin was increased by exogenous pST in all treatment groups (P < 0.05). Circulating NEFA

concentrations were higher for HC pigs compared to C and PF pigs on d 7 ($P < 0.01$). Exogenous pST increased NEFA concentrations in C ($P < 0.001$) and HC ($P < 0.02$) pigs. Circulating leptin was not different between treatment groups ($P > 0.29$). The mRNA abundance of IGF-I and the IGF-IR were not different between groups in liver or muscle tissue when expressed as a ratio to signal amplified from exogenously added rabbit globin mRNA. These results indicate that the ST/IGF-I system under basal conditions or stimulated with ST was not responsible for the growth reduction found in PRRSv and M. hyo. infected pigs.

INTRODUCTION

Despite sophisticated multi-site production systems and intensive biosecurity, the presence of diseases in the swine industry is extensive. The reductions in growth and costs associated with diseases have major economic consequences to livestock production. Respiratory diseases during the growing and finishing phases of growth are among the most costly to swine producers. Several infectious organisms are responsible for respiratory diseases in pigs, but the most frequently isolated organisms are *Mycoplasma hyopneumoniae* (*M. hyo*), porcine reproductive and respiratory syndrome virus (PRRSv) and swine influenza. In addition, respiratory diseases account for the greatest death losses in both nursery and grow-finish phases of growth (USDA, 2001).

The onset of a health-challenge from infectious diseases results in reduced growth performance, and in particular reduced muscle growth. The reductions in growth performance occur to preserve animal well-being and coordinate metabolism to support the immune system. However, despite the

frequency of disease, the regulation of growth in health-challenged animals is poorly understood.

Somatotropin (ST) is arguably the most important regulator of postnatal muscle growth in domestic animals. Somatotropin has direct affects on adipose tissue and indirectly mediates growth of skeletal muscle through the insulin-like growth factors (IGF) according to the original Daughaday model (Daughaday et al., 1972). Therefore, reductions in growth performance in response to a health-challenge are likely mediated at least partially by components of the ST/IGF axis.

MATERIALS AND METHODS

Animal Care. All procedures were approved by the Institutional Animal Care and Use Committee of North Carolina State University. Twenty-five barrows per replicate (two replicates) were weaned from sows at 10 d of age from North Carolina State University's (NCSU) Swine Educational Unit to an isolated specialized nursery building (Intensive Care Nurseries, INC. Colfax, IL). The early weaning was to ensure high health status of the pigs and to minimize the likelihood of infection and disease with *M. hyo*. Routine bi-annual serologic profiles indicated that the pig source did not test positive for *M. hyo* unil 40 to 60 d in the finishing phase. NCSU's Swine Educational Unit is a PRRSv negative herd. Barrows consumed manufactured liquid diet for nine d in the specialized nursery building, at which time they were moved to a conventional nursery building and raised in isolation until they weighed approximately 25 kg. During the isolation period, barrows were allowed to consume diets *ad libitum* and the diets met or exceeded NRC recommendations.

Fifteen closely matched pigs in body weight per replicate were selected and moved to isolated facilities at the NC State College of Veterinary Medicine for the remainder of the experiment. At this time, pigs were randomly assigned to one of the following groups: 1) non-infected, *ad libitum* consumption 2) infected with PRRS and *M. hyopneumoniae, ad libitum* consumption, and 3) non-infected, pair-fed according to the infected pigs. Pigs were housed in separate (infected vs. non-infected) environmentally controlled rooms in individual pens, fed a nutrient adequate standard corn-soybean meal based (Table 1) diet without medication, and had *ad libitum* access to water throughout the experiment. The rooms have a P-2 biohazard status and separtate air-handling systems.

After an adjustment period of 3 d, pigs were surgically fitted with indwelling jugular catheters. Briefly, pigs were denied access to feed for a period of 12 h prior to surgery. Pigs were sedated with ketamine (11 mg/kg BW) /xylazine (2 mg/kg BW) cocktail and then maintained on isoflourane gas anesthesia as a general anesthetic. The neck region was scrubbed three times with a Betadine solution and rinsed with a 70 % ethanol solution. A vertical incision (approximately 2.5 cm in length) was made approximately 2.5 cm from the midline immediately lateral to the larynx and blunt dissection was used to expose the external jugular vein. A small incision was placed in the vessels and a catheter (Microrenethane; 1200 mm x 1.6 mm I.D. x 3.2 mm O.D) was inserted to a depth of approximately five-cm. The catheter was anchored to the vessels and surrounding tissue with suture material and then exteriorized to the top of the shoulder. The exposed cavity was flushed with penicillin (Pen G) and closed with suture material. The incision site was treated with Nitrofuracin ointment. The hub

of the catheter was stored in an Elastikon pouch attached between the animal's shoulders.

For those pigs receiving the health-challenge, 9 mL of media containing approximately 10^6 *M. hyopneumoniae* (LI32 9-17-96, Strain 11)/mL was administered intratracheally during the catheterization procedure (Thacker et al., 1999). Non-infected pigs received an equal volume of sterile media, intratracheally. Eight d after surgery, infected pigs were given 2 mL/nare of 10^{4-5} $TCID_{50}$ PRRSv (isolate SD 23983) intranasally. The methods for administration of the two pathogens were previously established (Roberts and Almond, 2003). Non-infected pigs received 2-mL sterile media intranasally. The day of PRRSv innoculation was considered d 0 of the experiment. Refused feed was gravimetrically measured daily and pigs assigned to the non-infected, pair-fed group were given, in two equal portions, the amount of feed consumed on the previous day of the weight matched health-challenged pig. Pigs were gravimetrically weighed on a weekly basis.

Blood Collection and Analyses. A single blood sample was collected via jugular venipuncture on the day of surgery and *M. hyopneumoniae* infecion (d –8). Daily blood collections began 3 d prior to the PRRSv infection and continued for 3 wk after the infection. Fourteen d after infection, pigs within a treatment group (n=2 or 3 pigs depending on the replicate) were randomly assigned to receive 0 or 120 μg pST•kg body weight $^{-1}$•day $^{-1}$ (Reporcin solubilized in sterile water; Alpharma Co., Toorak, Australia) for 4 d. Blood samples were collected, immediately placed on ice, and then centrifuged (Beckman, model J-B6) at 824 x *g* for 10 min with plasma collected and frozen at -20° C until further analysis.

Plasma was analyzed in duplicate for urea nitrogen, insulin, IGF-I, NEFA, and leptin concentrations. Plasma urea nitrogen (PUN) concentrations were determined by the quantitative urease/Berthelot procedure (Sigma Diagnostics, St. Louis, MO) based on methods described by Fawcett and Scott (1960), and Chaney and Marback (1962). Plasma NEFA concentrations were determined by an enzymatic colorimetric method (Wako Pure Chimical Industries, Ltd., Richmond, VA). The sample mean for PUN pools was 11.1 $\pm$ 0.3 mg/dL and the intra- and inter-assay CVs were 3.1 and 6.0 %, respectively. The sample mean for NEFA pools was 204 $\pm$ 8 mEq/L and the intra- and inter-assay CVs were 3.0 and 7.9 %, respectively.

Plasma IGF-I, insulin, and leptin concentrations were analyzed by radio-immuno assays. Plasma IGF-I was dissociated from the IGF binding proteins (IGFBP) using the glycyl-glycine extraction method as described by Plaut et al. (Plaut et al., 1991). Recombinant human IGF-I was used for standards (Gropep, Ltd., Adelaide, Australia) and the radiolabeled ligand (Amersham Pharmacia Biotech UK Ltd., Buckinghamshire, England). Anti-human IGF-I polyclonal antiserum (GroPep) was used as the primary antibody and goat anti-rabbit gamma globulin (GroPep) was used as the second antibody. The sample means for IGF-I pools were 272 $\pm$ 15 ng/mL and the intra- and inter-assay CVs were 8.3 and 9.1 %, respectively. For the analysis of insulin, recombinant porcine insulin was used for standards and radiolabeled ligand (ICN Pharmaceutical, Inc., Costa Mesa, CA). Guinea pig anti-porcine insulin antibody was used as the primary antibody (ICN Pharmaceutical, Inc.). The sample means for insulin pools were 33.2 $\pm$ 1.5 μIU/mL and the intra- and inter-assay CVs were 7.0 and 5.7 %,

respectively. For the analysis of leptin, recombinant human leptin was used for standards and radiolabeled ligand (Linco Research, Inc., St. Charles, MO). Guinea pig anti-multi-species leptin antibody was used as the primary antibody (Linco Research, Inc.). These reagents were validated for use in the measurement of leptin in porcine serum in our laboratory (Whisnant and Harrell, 2002). The sample mean for the leptin pool was 1.63 ± 0.04 ng/mL and the intrassay CV was 4.3%.

Tissue Collection and Analyses. Animals were euthanized 4 d after the last pST injection (d 22) via Fatal Plus® (1 mL/4.5 kg BW) for the collection of tissues. Lungs were removed and assigned a visual consolidation score (Ross and Cox, 1988) as an assessment of pneumonia status (Table 2). The semitendinosus muscle and liver tissue were excised, immediately snap frozen in liquid nitrogen and stored at –80^{o}C until further analysis. Whole-cell (wcRNA) was extracted from muscle and liver tissue as described by Blondin et al. (Blondin et al., 2000). Briefly, TriReagent (1.1 mL/100 mg tissue; Molecular Research Center, Inc. Cincinnati, OH) was added to pre-weighed (~150 mg) frozen tissue in a 15 mL centrifuge tube and samples were briefly homogenized. Whole-cell RNA was extracted according to the manufacturer's protocol and resuspended in diethyl pyrocarbonate-treated water, with the exception that each sample was extracted a second time in TriReagent. The aqueous phase (RNA) of the first extraction was removed to a new tube and new TriReagent (1:1 v/v) was added to perform the second extraction. The concentration of wcRNA was determined based on absorbance at 260nm and the quality of wcRNA was verified by visualization of ethidium bromide stained 28S and 18S rRNA bands

on 1% agarose gels. RNA samples were diluted to 0.5 μg/μL and aliquots of wcRNA were snap frozen in liquid nitrogen and stored at -80°C until further analysis.

All samples were DNase treated and reverse transcribed as previously described (Crosier et al., 2002). Briefly, aliquots of wcRNA were thawed on ice and 4 μL (2μg wcRNA) were treated with 15 units of DNase I (Roche Molecular Biochemicals, Mannheim, Germany) for 20 min at 37°C. The reaction was stopped with 20 mM EDTA. Reverse transcription was conducted using random hexamers (Gibco BRL, Grand Island, NY) and SuperScript II reverse transcriptase (Gibco) using the manufacturer recommended conditions. Complimentary DNA (cDNA) was then purified with Qiaquick purification columns (Qiagen, Inc., Valencia, CA).

For the determination of the linear phase of amplification and optimal condition for each primer pair, polymerase chain reaction (PCR) was performed using cDNA from laboratory control porcine muscle and liver tissue. Reactions were conducted for 40 (Liver) or 45 (Muscle) cycles and tubes were removed every 5 cycles starting at 20 cycles. Polymerase chain reaction products were visualized on ethidium bromide-stained 1.5 % agarose gels. The signal intensity of individual bands was determined using the Optimas visual imaging system (BioScan Optimetric, BioScan Inc., Edmonds, WA). A response curve was generated for each primer pair (Figure 1) and the exponential phase of amplification was determined (Table 3).

Specific primer sequences, fragment size, and annealing temperature used for PCR amplification are given in Table 3. For each PCR reaction, 200 ng

of appropriate forward and reverse primers (custom synthesized by Sigma-Genosys, Woodlands, TX), 16 μM d-nucleotide triphosphates (dNTPs, Gibco), and 2.5 units of Taq polymerase (Roche) in a 20 μL reaction. In addition the reaction mixes included 2 μL of 10x PCR buffer (Roche) and 4 μL of Q solution (Qiagen, Inc., Valencia, CA). The reactions with IGF-I and rabbit globin primers contained 100-ng cDNA and the reactions with IGF-I receptor primers contained 150 ng cDNA. In addition to muscle or liver samples, each PCR assay contained a sample containing the master mix and no cDNA as a negative control and control cDNA at the beginning and end of the assay as positive controls. All PCR reactions were overlaid with oil and centrifuged at 13000 X g for 20 sec before being placed into a PTC-100 thermocycler (MJ Research Inc., Watertown, MA) at 92°C for 2 min. The standard PCR program used included denaturation for 30 sec at 94°C, 30 sec at the appropriate annealing temperature, and 60 sec at 72°C for primer extension.

Muscle and liver samples (n=8 to 10) were used for assessment of gene expression of IGF-I and the IGF type-I receptor. Each PCR assay was designed to assess expression of one mRNA type from a single tissue type and contained all experimental samples in duplicate. Samples were subjected to PCR amplification for the cycle number determined to be in the exponential phase (Table 3) and PCR products were visualized on an ethidium bromide stained 1.5 % agarose gel before the signal intensity of the resulting bands were determined. Intraassay coefficients of variation of duplicate samples for the liver and muscle samples for IGF-I and IGF-I receptor were 5.9, 4.6, 3.3, and 3.6 %, respectively. Potential variation between reverse transcription reactions was controlled

as described previously (Crosier et al., 2002) by adding 3 pg of exogenous rabbit globin mRNA to each wcRNA sample prior to reverse transcription. A separate PCR assay was performed to determine the amplification of rabbit globin cDNA and was used to calculate ratios of relative expression of mRNA expression for both IGF-I and IGF-I receptor.

Statistical Analysis. Data were subjected to analysis of variance using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). Data were evaluated for the effects of treatment, pST, replication, and all appropriate interactions. The effect of treatment (control, health challenged, or pair-fed) and pST (0 vs 120 μg pST•kg body weight $^{-1}$•day $^{-1}$) response were contrasted using a protected least significant difference test (Steel et al., 1997). The experimental unit for all analyses was individual pig. The significance level for all tests was set at $P < .05$.

RESULTS

Growth Performance. Initial body weight did not differ (33.9 ± 1.0; $P > 0.64$) among treatment groups (Figure 4). Body weight was unaffected by treatment on d 7, but control pigs were heavier than both the pair-fed and health challenged groups on d 14 (46.9 ± 1.4 vs. 43.2 ± 1.3 and 43.4 ±1.6, respectively; $P < 0.10$) and 21 (53.2 ± 1.4 vs. 45.8 ± 1.8 and 46.8 ± 1.5, respectively; $P < 0.01$). From d 0 to 21, control pigs gained 919 ± 31 g/d compared to 601 ± 33 and 678 ± 40 for pair-fed and health challenged pigs, respectively (Figure 4; $P < 0.01$). The effect of the health challenge on ADG was most prominent from d 14

to 21, when pair-fed (431 ± 50 g/d) and health challenged (393 ± 68 g/d) pigs gained approximately 52 % slower than control pigs ($P < 0.01$). No difference in feed intake was observed during the collection period before PRRSv inoculation (Figure 3; $P > 0.68$). Infection with PRRSv and *M. hyo.* reduced feed intake beginning on d 7 of infection ($P < 0.04$), with a maximum reduction of 54 % (2.0 ± 0.09 vs 0.93 ± 0.14 kg; $P < 0.001$) on d 16. Disease status did not affect feed conversion (G:F) except from d 14 to 21 where the health challenged group had a lower G:F ratio compared to the control group (Figure 6; $P < 0.01$). However, the pair-fed group (0.477 ± 0.039) had a 26 and 33 % lower G:F compared to control (0.645 ± 0.039) and health challenged (0.719 ± 0.036) pigs, respectively, from d 0 to 7 of treatment ($P < 0.02$). No other treatment differences were observed for feed conversion ($P > 0.10$).

Plasma analysis. Basal circulating IGF-I levels were 322 ± 22 ng/mL, regardless of treatment (Figure 7; $P > 0.15$) from d –8 to d 13 of treatment. The administration of pST increased circulating IGF-I 2.5-fold after 4 d of injections (Figure 8; $P < 0.01$), regardless of treatment. However, after the first pST injection (d 15) the response to pST was greater ($P < 0.01$) in the control group compared to the pair-fed group, and the disease challenged pigs' response was intermediate. Basal PUN concentrations averaged 10.0 ± 0.5 mg/dL, regardless of treatment (Figure 9; $P > 0.50$). The only difference in basal PUN was observed on d 11 after PRRSv inoculation, when the control group had a higher PUN concentration compared to the pair-fed group ($P < 0.05$). Plasma urea nitrogen concentration of the disease challenged pigs was intermediate and was not different than the control or pair-fed group on d 11 ($P > 0.15$).

Exogenous pST reduced ($P < 0.001$) PUN concentrations approximately 28 %, regardless of treatment group (Figure 10; $P > 0.90$). Circulating insulin concentrations averaged 23.2 ± 2.9 μIU/mL on d –1 and 7, regardless of treatment (Figure 11; $P > 0.49$). However, on d 14 after PRRSv infection, the control group had a higher plasma insulin concentration, compared to the disease challenge group ($P < 0.02$). The pair-fed group was intermediate and was not different from either the control or disease challenged group ($P > 0.12$). Insulin concentrations were increased by exogenous pST in all treatment groups (Figure 12; $P < 0.05$), but tended to increase ($P < 0.10$) to a greater extent in the control group (16.9 ± 2.1) compared to the pair-fed group (11.6 ± 2.0). The disease challenged group was intermediate and was not different compared to either control or health challenged pigs ($P > 0.19$). Circulating NEFA concentrations did not differ on d –1 (Figure 13; $P > 0.7$), but were higher for the disease challenged group compared to the control and pair-fed groups on d 7 ($P < 0.01$; 204 ± 12 vs 150 ± 12 and 157 ± 11 μEq/L, respectively). By d 14, no difference in circulating NEFA concentrations were observed between any treatment group ($P > 0.11$). Exogenous pST increased circulating NEFA concentrations in the control ($P < 0.001$) and health challenged ($P < 0.02$) groups, but not in the pair fed group ($P > 0.37$). Circulating leptin concentrations averaged 2.4 ± 0.2 ng/mL for the entire treatment period and were not different between treatment groups ($P > 0.29$).

Semiquantitative RT-PCR Assay. The various primer sequences, annealing temperatures, product lengths, and cycle numbers used for the individual PCR assays are listed in Table 3. Expression of mRNA for IGF-I or the

IGF-I type I receptor in the liver was not affected by treatment ($P > 0.20$). However, the expression of mRNA for both IGF-I and its receptor were increased in semitendinosus muscle of pair-fed and disease challenged pigs, compared to the control group ($P < 0.02$). The expression of mRNA for exogenous rabbit globin did not differ between treatment groups ($P > 0.45$). Similarly to the analysis of unadjusted amplification signal intensities, the expression of mRNA for IGF-I and the type-I receptor in liver was similar between treatment groups ($P > 0.17$) when amplification signals were expressed as a ratio to signals amplified from exogenous rabbit globin. However, when adjusted to rabbit globin, no difference was observed in the mRNA expression of IGF-I and the type-I receptor between treatment groups ($P > 0.20$).

DISCUSSION

At necropsy, lung lesions indicative of a M. *hyo.* challenge were only observed in health challenged pigs, indicating that our early weaning program and health challenges were effective (Table 2). In addition, all pigs were seronegative for PRRSv at d 0 and PRRSv titers were present only in infected pigs at the termination of the experiment, confirming that the source of pigs was a PRRSv negative herd.

Exposure to high levels of antigens activates the immune system and results in a cascade of physiological adjustments to preserve animal well being (see review by Johnson, 1997; Webel et al., 1997). The physiological adjustments include reduced feed intake and growth rate, and in particular reduced muscle growth. The immune response (i.e. cytokines; tumor necrosis

factor alpha) is implicated in the decrease in growth performance and feed intake observed during an immune challenge (Johnson 1997). In the current experiment, overall ADG was reduced by 26 % by infection with PRRSv and *M. hyo.*, and the reduction in growth was maximized at 52 % from d 14 to 21 of treatment. No difference in ADG was observed between the infected and pair-fed groups, indicating that a reduction in feed intake may be predominantly responsible for the reduction in growth. Feed intake in the current experiment was markedly reduced in pigs infected with PRRSv and *M. hyo*. Beginning on d 5 of treatment, disease challenged pigs consumed less feed and this reduction was observed throughout the rest of the experiment. These data are similar to published reports of the efficacy of PRRSv and *M. hyo.* on reducing growth performance of growing swine (Roberts and Almond, 2003). The current experiment, as well as Roberts and Almond, (2003), utilized direct infection with PRRSv and *M. hyo.*, but Williams et al. (1997) achieved chronic immune system activation by nonspecific environmental pathogens. Similarly to the current study, Williams et al. (1997) observed a reduction in feed intake and growth rate, although the reduction was less pronounced than in the current experiment

Plasma urea nitrogen concentrations are an indirect measure of the extent of amino acid oxidation, and circulating NEFA is an indirect measure of lipolysis and(or) fatty acids available for uptake into tissues. Basal (d –8 to 14) PUN concentrations were not different among treatment groups, except on d 11 where the pair-fed pigs had lower PUN concentrations compared to controls. Lower PUN concentrations were expected in the pair-fed and health challenged groups because of the reduction in feed intake. Because of the drastic reduction in

growth rate in conjunction with decreased feed intake, the magnitude of the reduction in PUN concentrations was limited, which suggests that the efficiency of amino acid use was not affected by the health challenge. Exogenous pST decreased PUN approximately 25 % in the current experiment, regardless of treatment. This observation agrees with previous research, in that exogenous pST increased lean tissue deposition, thus decreased the oxidation of dietary amino acids (Campbell et al., 1989; Campbell et al., 1990).

On d 7, circulating NEFA concentrations were approximately 36 % higher in pigs infected with PRRSv and *M. hyo.* compared to control and pair-fed pigs. Brown et al. (2001) observed that pigs infected with PRRSv and *M. hyo.* contained less total body fat compared to controls, indicating that infected pigs had either a higher rate of lipolysis or a decreased rate of lipogenesis. The higher plasma NEFA concentrations in the current experiment agree with this observation, in that circulating NEFA concentrations are increased when lipolysis is increased or lipogenesis is decreased. Exogenous pST increased circulating NEFA concentrations approximately 30 % in control pigs in the current experiment, but not in the pair-fed pigs, and only a modest increase (15 %) was observed for the disease challenged pigs. The effect of pST on circulating NEFA in the control pigs was expected because of the decrease in lipogenesis associated with the administration of exogenous pST (Dunshea 1993). It is possible that the lack of response in the pair-fed group and modest effect of the health challenge group are due to a preceding down regulation of key lipogenic enzymes. The activity of fatty acid synthase and acetyl-CoA carboxylase are reduced in adipose tissue from pST treated pigs (Magri et al., 1990; Harris et al.,

1993; Donkin et al., 1996).

Circulating insulin concentrations are predominately regulated by plasma glucose availability. In the current experiment, no differences in circulating insulin concentrations were observed between treatment groups on d –1 or d 7. However, plasma insulin concentrations were higher in the control pigs compared to the disease challenged pigs on d 14. Because of the difference in feed intake in pigs infected with PRRSv and *M. hyo.*, circulating glucose concentrations should be lower in infected pigs, thereby leading to a lower insulin concentration, as seen on d 14. Circulating insulin concentrations were increased for all treatment groups in response to exogenous pST. This response is consistent with previous research (Dunshea et al., 1992). The response to exogenous pST tended to be greater for the control group compared to the pair-fed group. This may be explained by a reduction in glucose available for growth resulting from lower feed intake.

Leptin has been implicated, in addition to the cytokines, in the acute inflammatory response. Because leptin has a negative impact on feed intake and stimulates thermogenesis, it is possible that leptin plays a role in the anorectic and metabolic changes induced by an immunological challenge. Grunfeld et al. (1996) reported increased circulating leptin concentrations and adipose tissue leptin mRNA levels in hamsters during the host response to an endotoxin challenge. In addition, Sarraf et al. (1997) found similar increases in leptin levels with administration of pro-inflammatory cytokines. In contrast to rodent data, Leininger et al. (2000) reported a reduction in leptin expression in pigs after an acute challenge with endotoxin. No differences in circulating leptin

concentrations were observed in the current experiment. Our data agree with Spurlock et al. (1998), who reported no changes in leptin expression in pigs after an acute endotoxin challenge. Circulating leptin was also unaffected by exogenous pST in pigs in the present study. However, Oliver et al. (Chapter 3) reported that exogenous pST administration transiently increased circulating leptin concentrations by two h post pST treatment, but returned to baseline by six h post treatment. Also, circulating levels of leptin were lower at 12, 24, 48, and 56 h after the initial daily pST injection, compared to baseline values. Due to the transient increase of the circulating leptin concentrations to exogenous pST, changes in plasma leptin may not have been observed in the present study due to the time of blood sampling.

Most studies examining the effects of a health-challenge on the regulation of the ST/IGF system utilize a challenge of the *Escherichia coli* lipopolysaccharide (LPS), which results in acute anorexia, fever, and catabolic affects on metabolism. Administration of LPS to rats resulted in decreased circulating IGF-I and ST concentrations within 4 h (Fan et al., 1994; Soto et al., 1998). Similarly to rats, administration of LPS to humans (Lang et al., 1997), swine (Hevener et al., 1997), and sheep (Briard et al., 2000) resulted in a reduction in circulating IGF-I. In contrast, circulating IGF-I concentrations in the current experiment were not reduced by PRRSv and *M. hyo.* challenge (Figure 7). In addition, no differences were observed in the response of circulating IGF-I to exogenous pST (Figure 8). Similar to previous studies, plasma IGF-I was increased by exogenous pST approximately 2.5-fold (Daughaday et al., 1972; Dunshea et al., 1992; Harrell et al., 1999), regardless of treatment group. The

lower circulating IGF-I levels in rats challenged with LPS are associated with decreased production of IGF-I, measured by lower mRNA abundance of liver IGF-I (Fan et al., 1994) and lower perfused liver output of IGF-I (Fan et al., 1995). However, in the current experiment, no difference in liver IGF-I mRNA abundance was observed. These data agree with our observation that plasma IGF-I is unaffected by PRRSv and *M. hyo.*, because the majority of circulating IGF-I is secreted by the liver and liver mRNA abundance is highly correlated with circulating IGF-I abundance (Fan et al., 1994). In addition, no difference was observed for the expression of IGF-I type-I receptor mRNA in the liver. Similarly to the analysis of unadjusted amplification signal intensities, the expression of mRNA for IGF-I and the type-I receptor in liver was similar between treatment groups when amplification signals were expressed as a ratio to signals amplified from exogenous rabbit globin. The lack of a response to the disease challenge in the liver may indicate that the sensitivity of skeletal muscle to circulating IGF-I is reduced in the infected pigs to account for slower growth rates. Muscle IGF type-I receptor mRNA abundance was increased in pigs infected with PRRSv and *M. hyo.*, as well as pigs pair-fed to the health challenged pigs. However, due to the numerical difference in rabbit globin mRNA abundance in muscle (Figure 17), no difference in muscle IGF type-I receptor mRNA abundance was observed when amplification signals were adjusted to the mRNA abundance of exogenous rabbit globin. Similarly to IGF type-I receptor mRNA, the unadjusted abundance of IGF-I mRNA in semitendinosus muscle was increased in pair-fed and disease challenged pigs. Again, when expressed as a ratio of relative expression to rabbit globin mRNA, IGF-I mRNA abundance was not different between

treatment groups. Collectively, these data suggest that infection with PRRSv and *M. hyo.* do not affect the abundance of mRNA for IGF-I or its receptor in liver or muscle.

IMPLICATIONS

The results from this experiment show that the ST/IGF-I system was not responsible for the growth reduction found in growing pigs infected with porcine reproductive and respiratory syndrome virus and *M. hyopneumoniae.* Circulating IGF-I, as well as liver and muscle IGF-I mRNA, did not differ between treatment groups, in spite of a 50 % decrease in growth performance. In addition, opportunities may exist to utilize exogenous pST to improve performance in chronically disease challenged pigs. Circulating IGF-I was increased and PUN was decreased in all treatment groups in the current study, indicating that the ST/IGF-I system in active in the chronically disease challenged pigs.

Table 1. Composition and calculated analysis of the diet

	%, as fed basis[a]
Ingredient	
Corn	70.2
Soybean meal	23.1
Poultry fat	4.0
Dicalcium phosphate	1.25
Limestone	0.9
Salt	0.35
Vitamin-Mineral premix[a]	0.25
Calculated Analysis	
ME, Mcal/kg	3.41
Crude protein, %	17.3
Lysine, %	0.94
Calcium, %	0.78
Total Phosphorous, %	0.61

[a]Vitamin/Mineral premix (ADM, Des Moines, IA) contributed the following nutrients per kg of diet: 0.3 mg selenium as sodium selenite, 110 mg zinc as zing oxide, 1875 IU Vitamin A as Vitamin A acetate in gelatin, 1375 IU Vitamin D_3, 22 IU Vitamin E as menadione sodium bisulfate, 0.25 mg calcium as calcium carbonate, 22 mg copper as copper sulfate, 110 mg iron as orin sulfate, 55 mg manganese as manganous oxide, 0.3 mg iodine as ehlendiamine dyhydroiodide, 165 mg choline as choline chloride, 22 mg niacin, 17.6 mg pantothenic acid as d-calcium pantothenate, 0.02 mg Vitamin B_{12}, and 4.4 mg riboflavin.

Table 2. Results of diagnostic tests for verification of PRRSv and *M. hyopneumoniae (M. hyo.)* infection.

	PRRSv and M. hyo. ELISA test positive[a] (%)		Lung Scores[b] (%)					
Treatment	d 0	d21	0	1-10	11-20	21-30	31-40	41-50
Control	0	0	10	0	0	0	0	0
Challenged	0	100	0	0	2	3	1	2
Pair-fed	0	0	9	0	0	0	0	0

[a]Reported at the percent of pigs testing positive for PRRSv and *M.hyo.* on either d 0 or d 21 of treatment. For PRRSv, an S:P of 0.2 was considered positive.
[b]Reported as percent lung consolidation. Lung lesions were scored as described by Ross and Cox (1988) during necropsy on d 22 after PRRSv infection (d 30 after M. *hyopneumoniae* infection).

Table 3. Primer sequences used for PCR analysis of porcine liver and semitendinosus muscle.

Primer	Sequence	Fragment Size (bp)	Annealing Temperature (°C)	Cycle number
IGF-I[a]	5'-GGAGACAGGGGCTTTTAT 5'-ACTCCCTCTACTTGTGTT	239	55	23
IGF-Ir[b]	5'-ATGCTGTTTGAACTGATGCGCA 5'CCGCTCGTTCTTGCGGCCCCCG	354	55	28
Globin[c]	5'-GCAGCCACGGTGGCGAGTAT 5'-GTGGGACAGGAGCTGAAAT	257	65	25

[a]Insulin-like growth factor I; sequence was generated utilizing Genebank mRNA seqences and Oligo 4.0.2 primer analysis software (Plymouth, MA).
[b]IGF Type I receptor; sequence obtained from Ge et al. (2000).
[c]Rabbit globin; sequence obtained from Temeles et al. (1994).

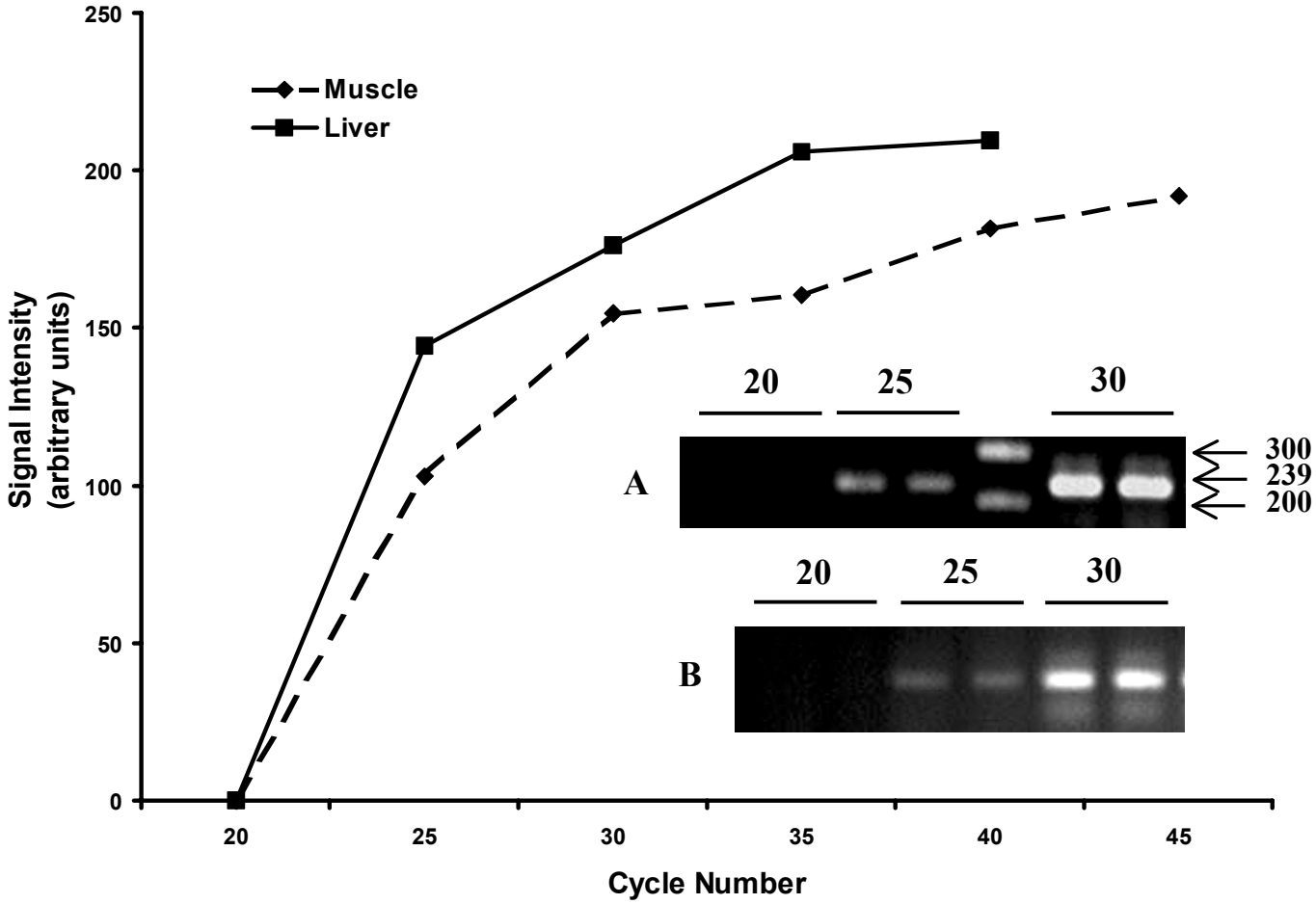

Figure 1. Inset: A representative ethidium bromide-stained agarose gel depicting PCR products in duplicate from cycles 20, 25 and 30 for liver (A) and muscle (B) using IGF-I primers used to determine the exponential phase of amplification. Graph: Data based on complete analysis of the average signal intensities for the duplicate PCR products for the IGF-I primer pair and muscle and liver control cDNA samples. The SEM of each duplicate ranged from 0 to 5 %. Cycle number 23 was determined to be within the exponential phase of amplification for both muscle and liver and was utilized for all subsequent PCR reactions using the IGF-I primer pair.

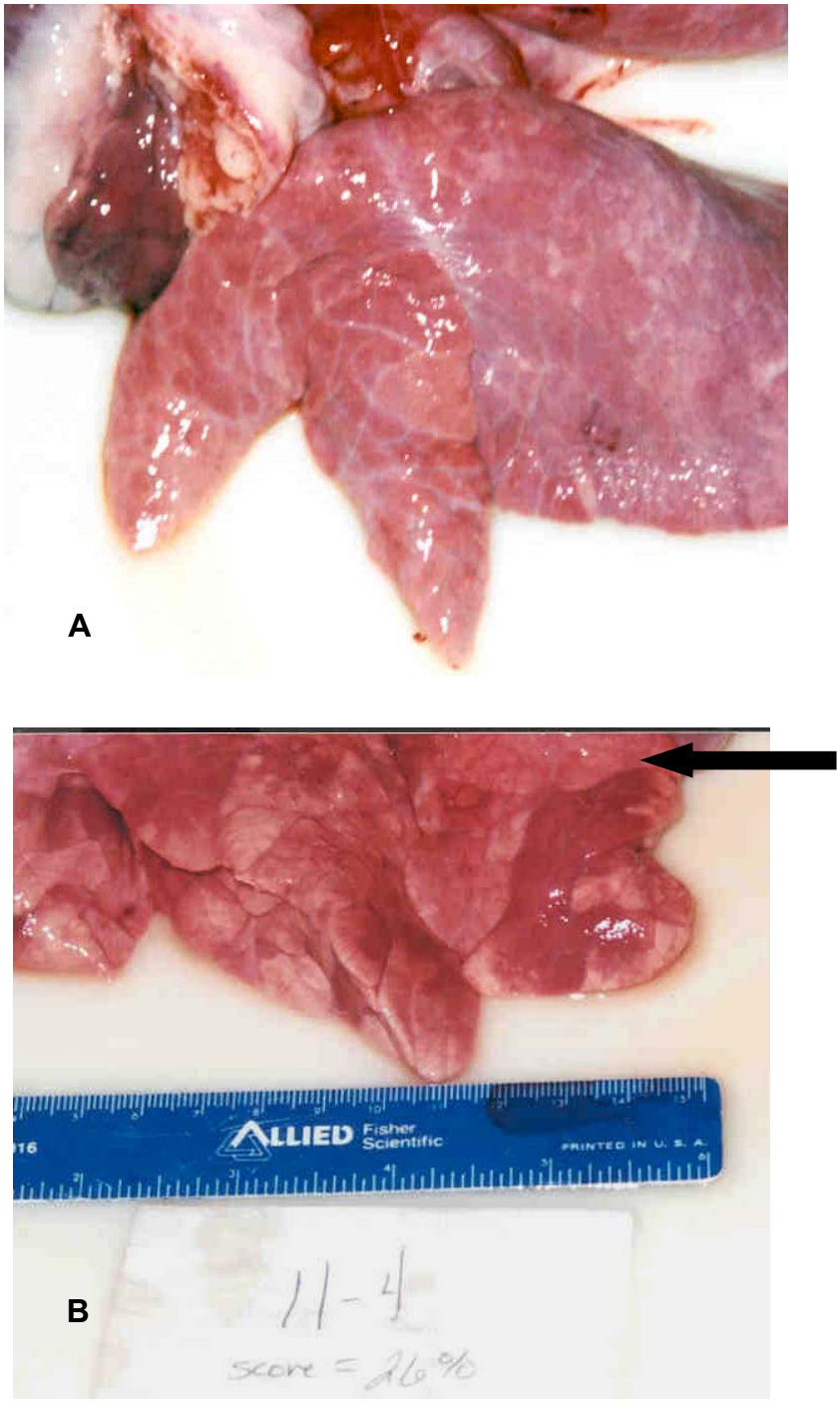

Figure 2. Lung representative of healthy (Panel A) or infected (Panel B) pigs with gross pathological lesions consistent with pneumonia caused by PRRSv and *M. hyopneumoniae*. The arrow in Panel B represents the line of demarcation between healthy (light) and pneumonic (dark) tissue.

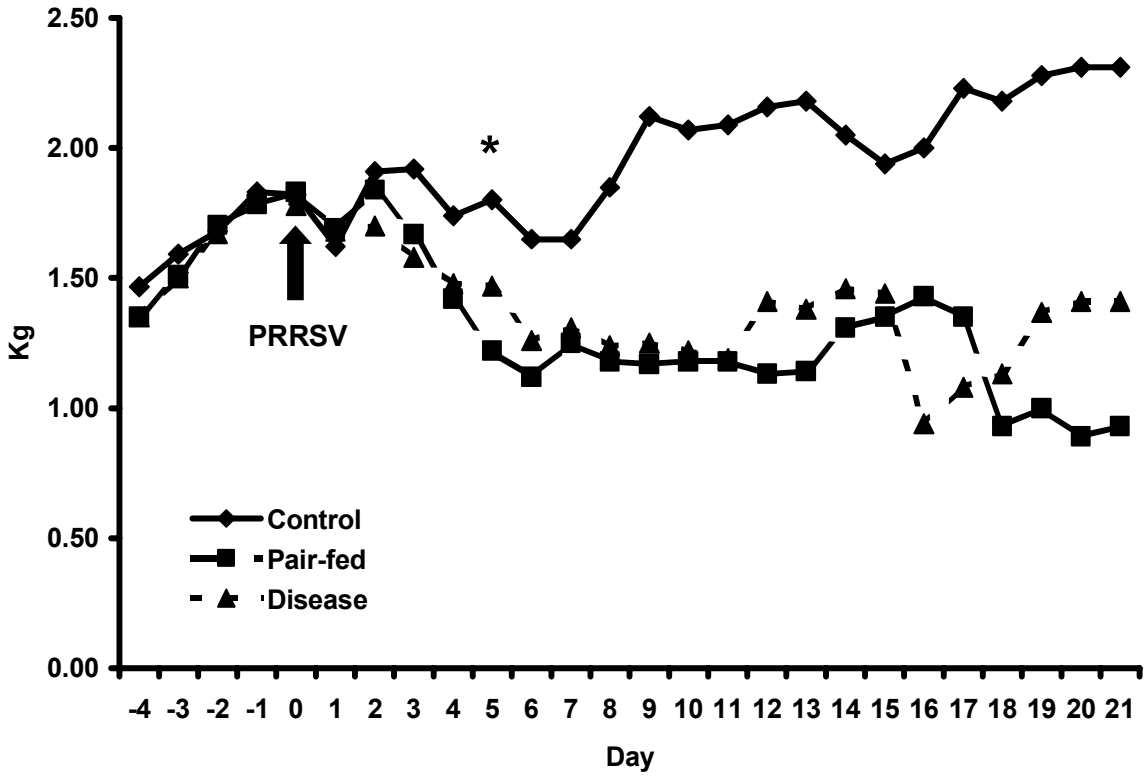

Figure 3. Effect of health status on average daily feed intake (ADFI) of pigs prior to infection up to 21-d post infection. Values shown are least squared means and the pooled SEM was 0.04. ADFI was decreased ($P < 0.05$) by disease challenge beginning on d 5 and continued to be lower until d 21. n = 8 to 10.

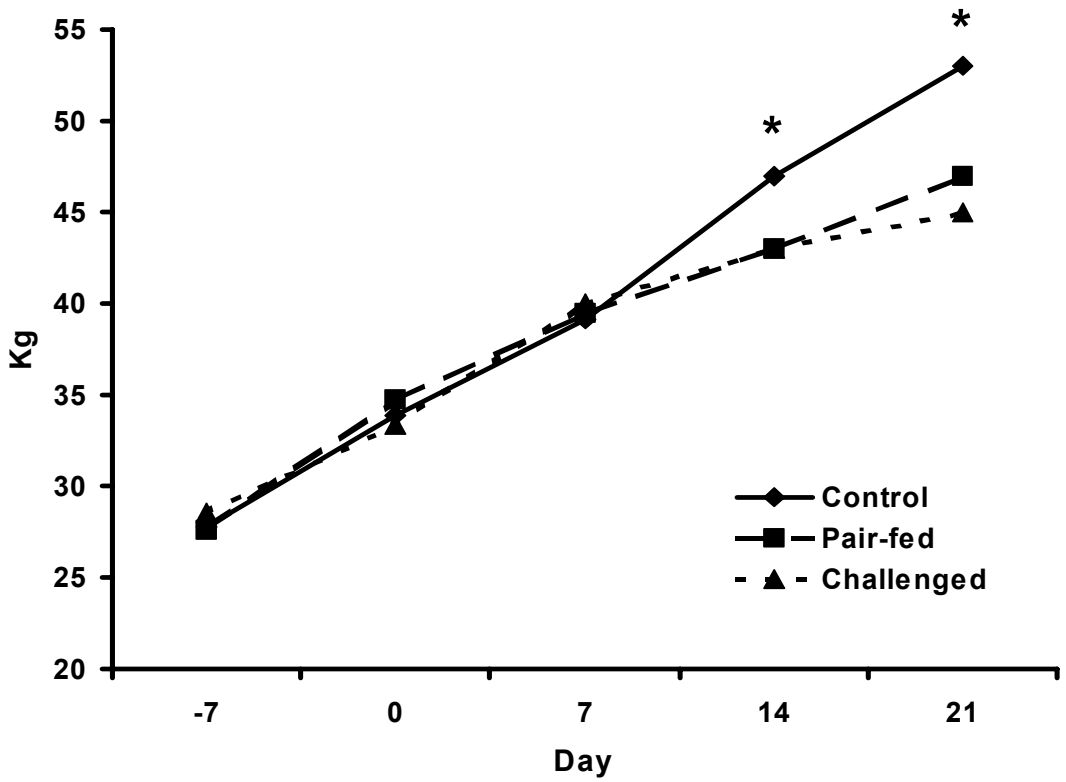

Figure 4. Effect of health status on live weight of pigs prior to infection up to 21-d post infection. Values shown are least square means and the pooled SEM was 0.07. Live weight was decreased ($P < 0.05$) by disease challenge beginning on d 14 ($P < 0.10$) and d 21 ($P < 0.05$). n = 8 to 10.

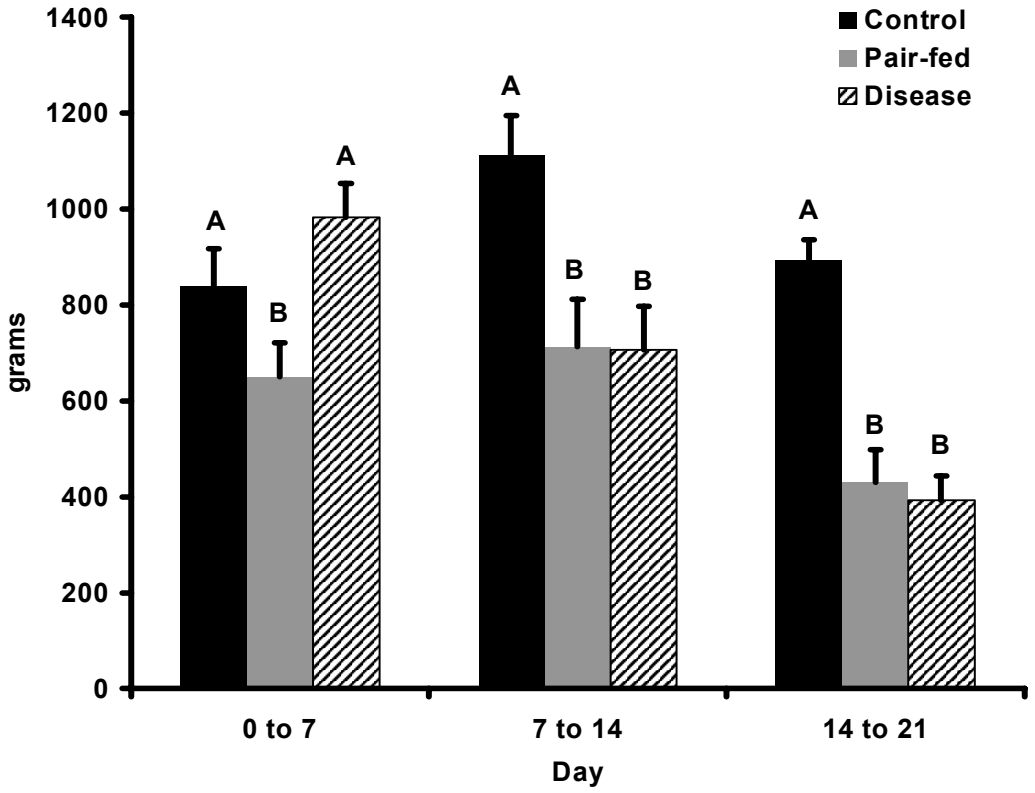

Figure 5. Effect of health status on average daily gain of pigs from d 0 to 7, 7 to 14 and 14 to 21 postinfection. Values shown are least square means ± SEM. Means lacking a common superscript, within a period, differ ($P < 0.05$). n = 8 to 10.

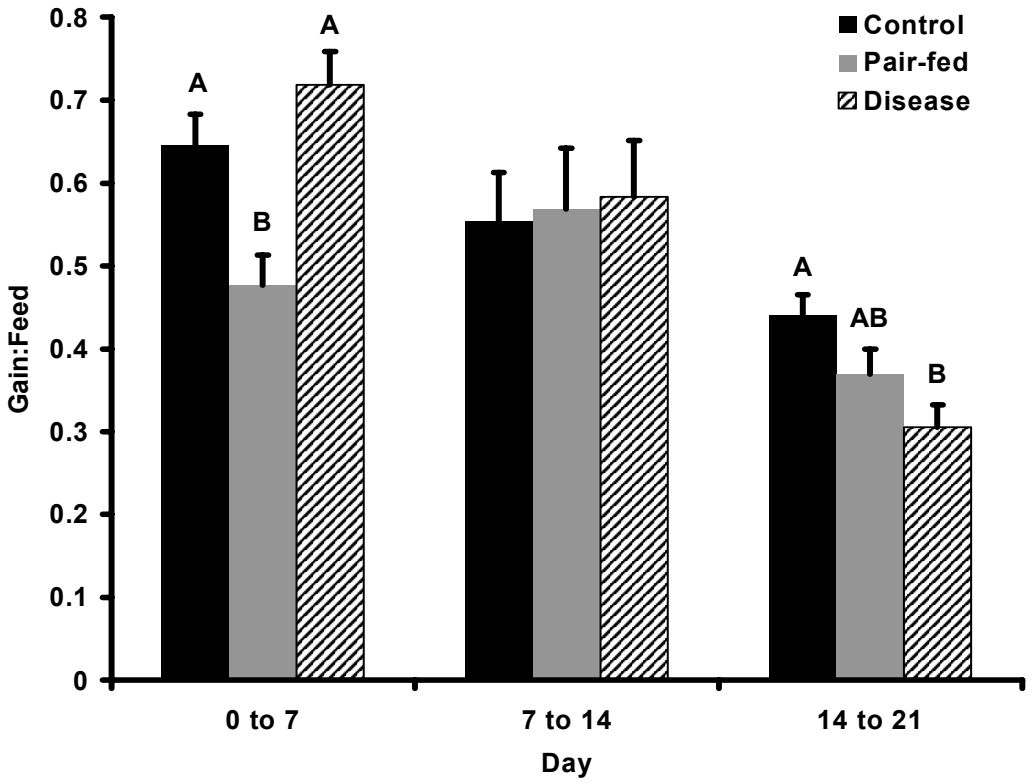

Figure 6. Effect of health status on feed efficiency (Gain:Feed) of pigs from d 0 to 7, 7 to 14 and 14 to 21 postinfection. Values shown are least square means ± SEM. Means lacking a common superscript, within a day, differ($P < 0.05$). n = 8 to 10.

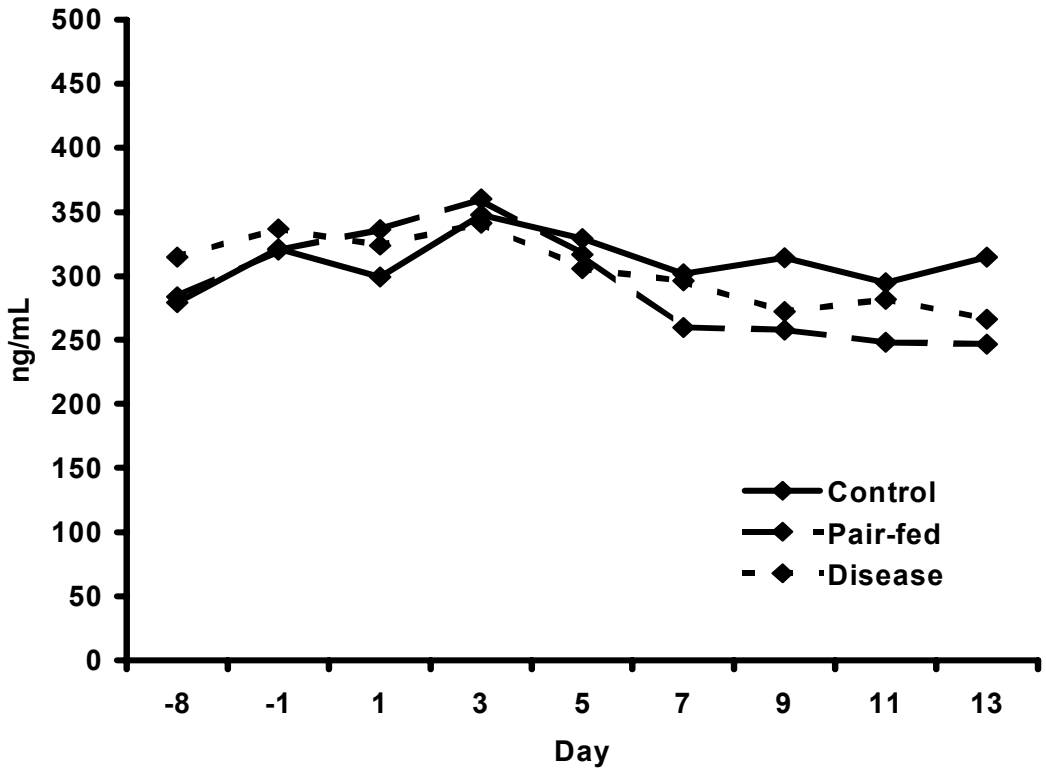

Figure 7. Effect of health status on basal (d –8 to d 13) circulating IGF-I concentrations. Values shown are least square means and the overall SEM was 6.8. Health status had no affect on basal circulating IGF-I. n = 8 to 10.

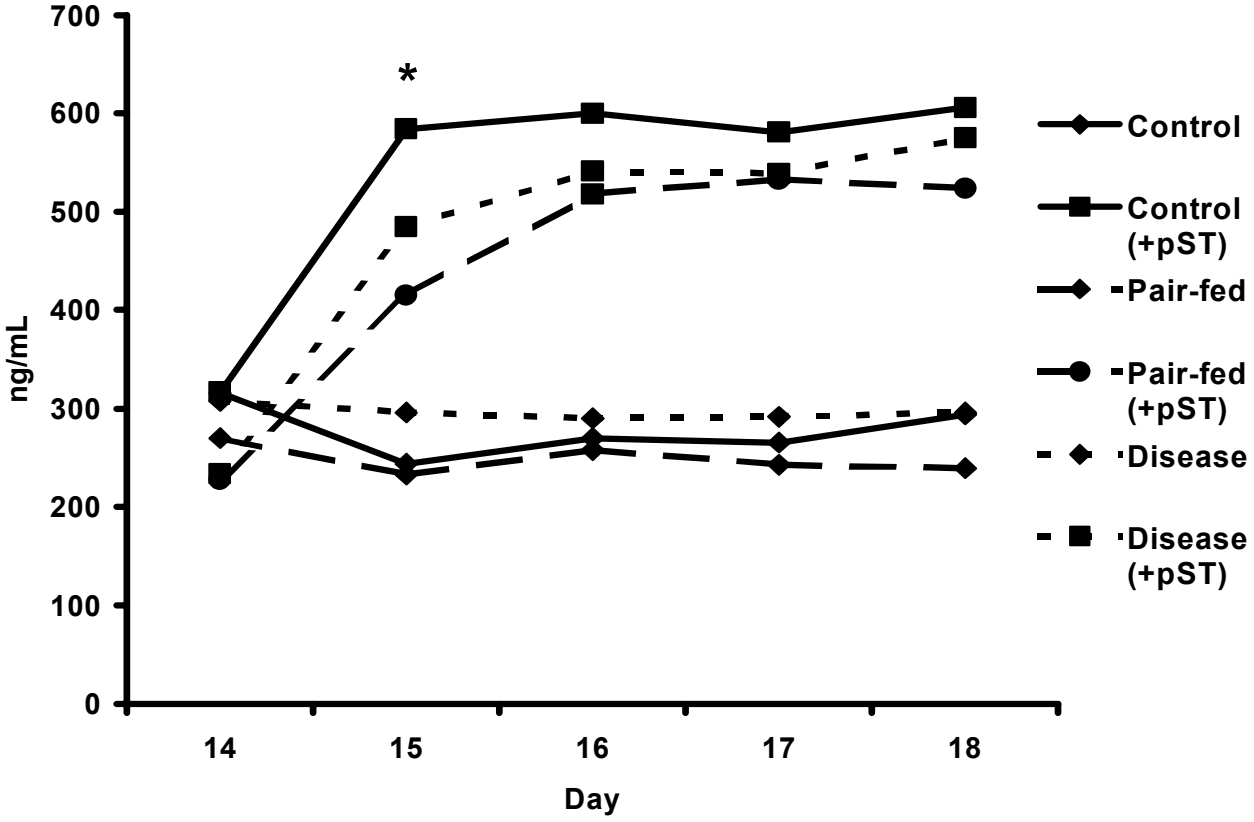

Figure 8. Effect of health status on pST stimulated (d 14 to 18) circulating IGF-I concentrations. Values shown are least square means and the overall SEM was 15. *Control(ST) > Pair-fed(ST); *P* <0.05. n = 3 to 5.

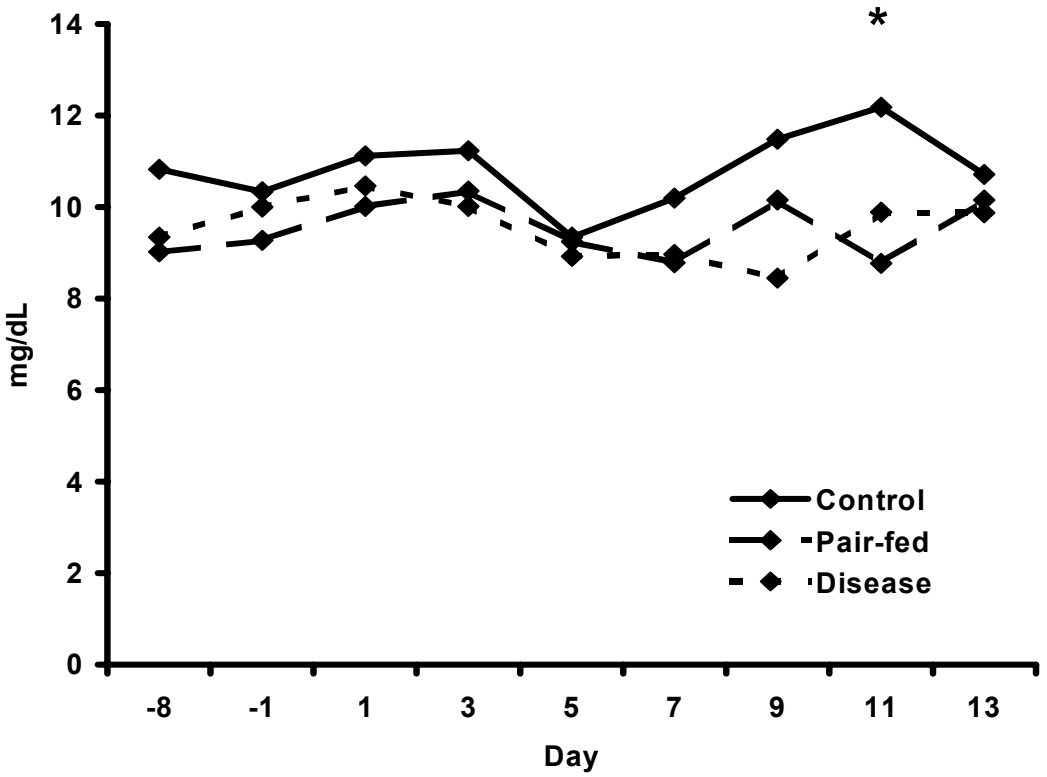

Figure 9. Effect of health status on baseline (d –8 to d 13) plasma urea nitrogen (PUN) concentrations. Values shown are least squared means and the overall SEM was 0.5. Health status had no affect on basal PUN, except on d 11 where the control pigs had higher PUN concentrations than pair-fed pigs (P <0.05). n = 8 to 10.

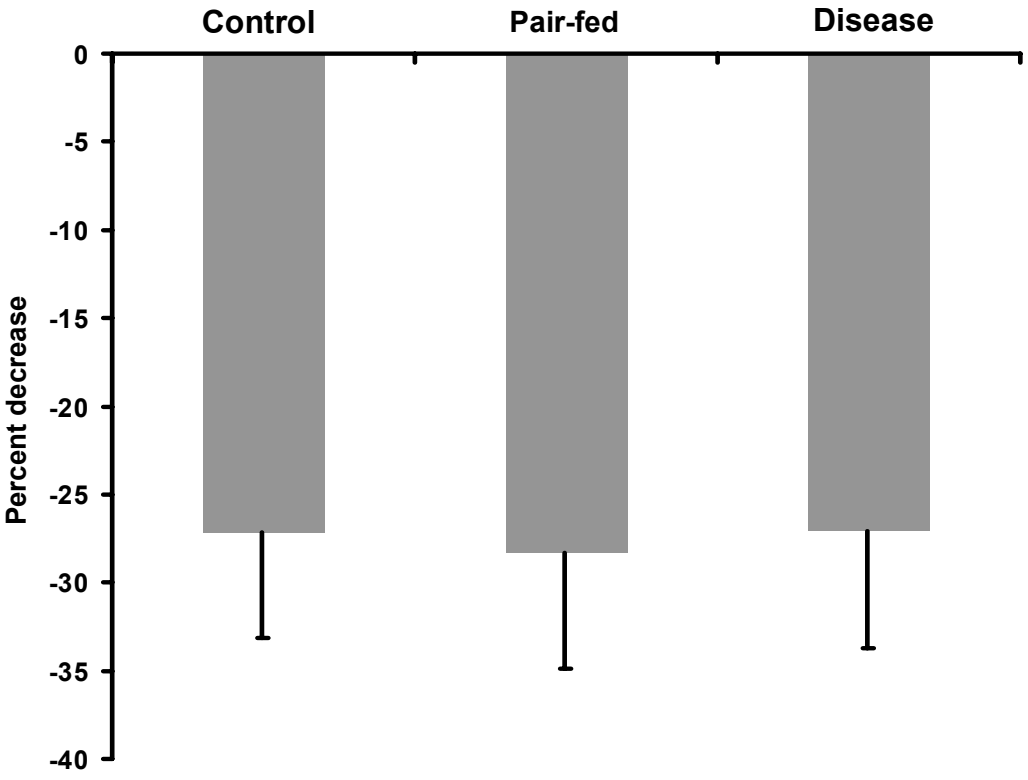

Figure 10. Effect of health status on the pST stimulated (d 14 to 18) change in plasma urea nitrogen (PUN) concentrations. Values shown are least square means ± SEM. Exogenous pST decreased PUN (P < .01), regardless of treatment group. n = 3 to 5.

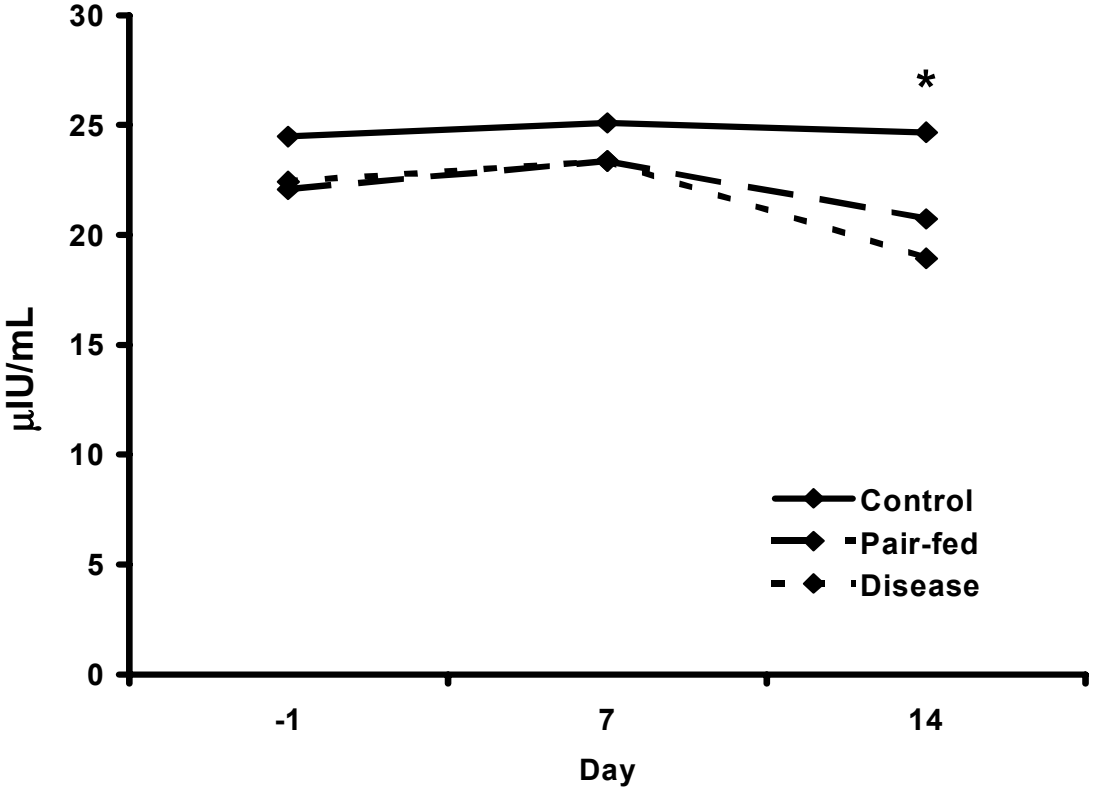

Figure 11. Effect of health status on basal (d –8 to d 13) insulin concentrations. Values shown are least squared means and the overall SEM was 1.3. Health status had no affect on basal inculin concentration, except on d 14 where the control pigs had higher insulin concentrations than disease challenged pigs ($P < 0.02$). n = 8 to 10.

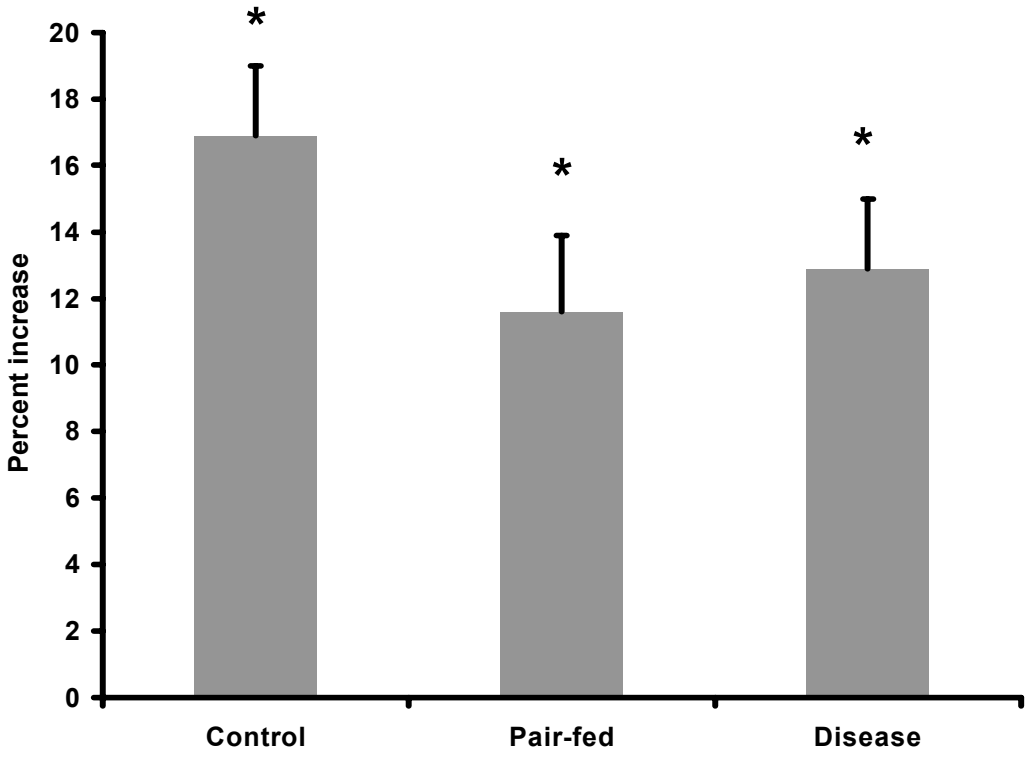

Figure 12. Effect of health status on the pST stimulated (d 14 to 18) change in insulin concentrations. Values shown are least square means ± SEM. Exogenous pST increased insulin concentrations in all treatment groups (P < .01), but insulin concentrations tended to increase to a greater extent in control pigs, compared to the pair-fed pigs (P < .10). n = 3 to 5.

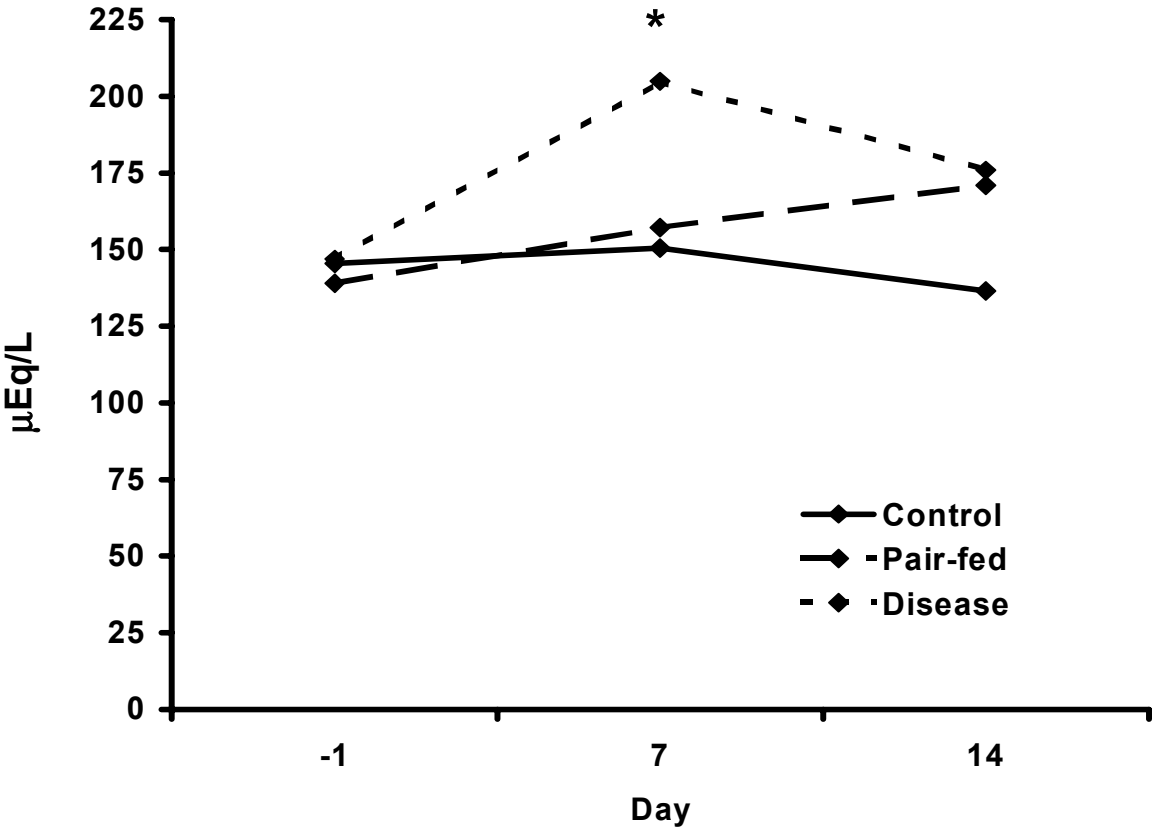

Figure 13. Effect of health status on basal (d –8 to d 13) NEFA concentrations. Values shown are least square means and the overall SEM was 13. Health status had no affect on basal NEFA concentrations, except on d 7 where the health challenged pigs had higher NEFA concentrations than control pigs (P < 0.01). n = 8 to 10.

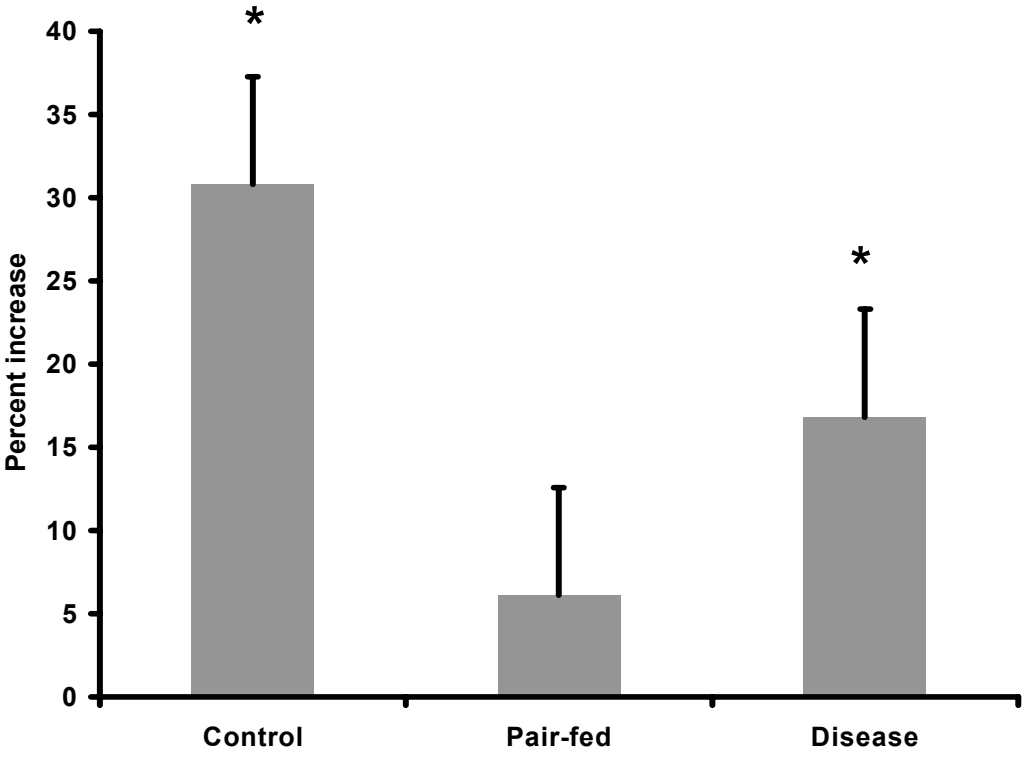

Figure 14. Effect of health status on the pST stimulated (d 14 to 18) change in NEFA concentrations. Values shown are least square means ± SEM. Exogenous pST increased NEFA concentrations in control and disease challenged pigs ($P < .01$), but not in pair fed pigs ($P > .31$). n = 3 to 5.

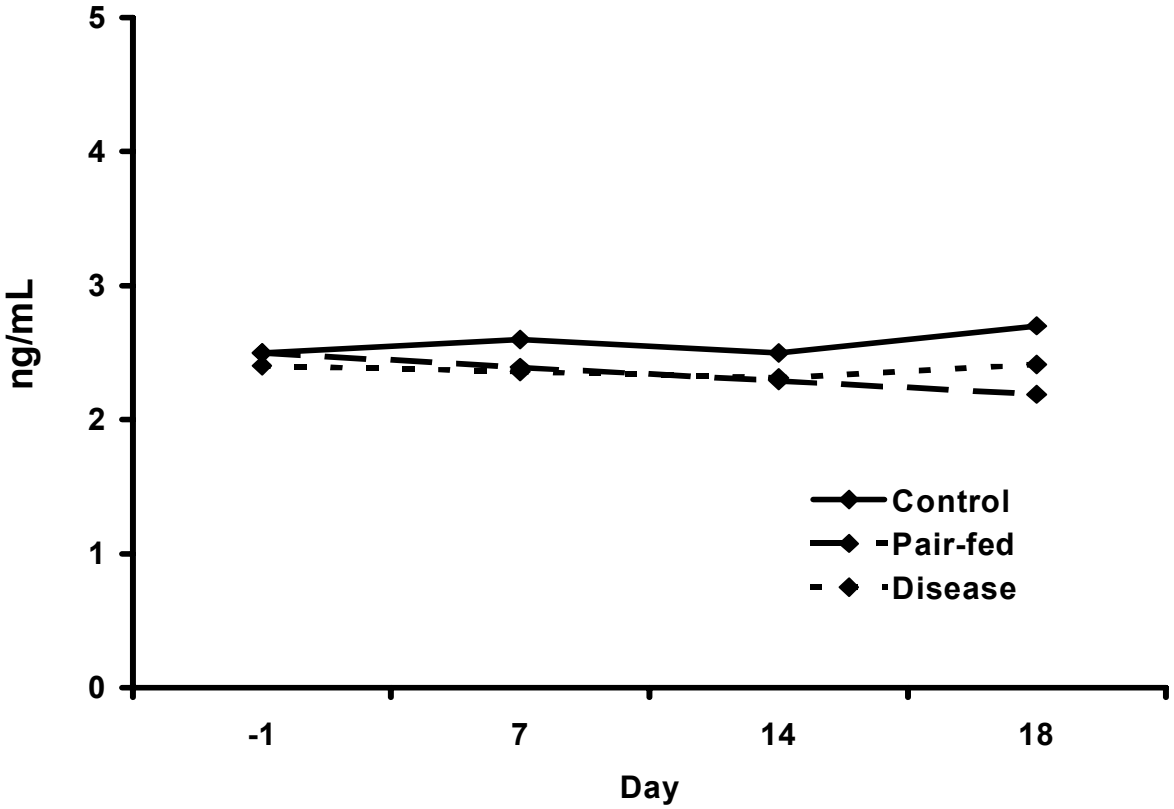

Figure 15. Effect of health status on circulating leptin concentrations from d –1 to 18 of treatment. Values shown are least square means and the overall SEM was 0.2. Health status had no affect circulating leptin concentrations, regardless of treatment ($P > .05$). n = 8 to 10.

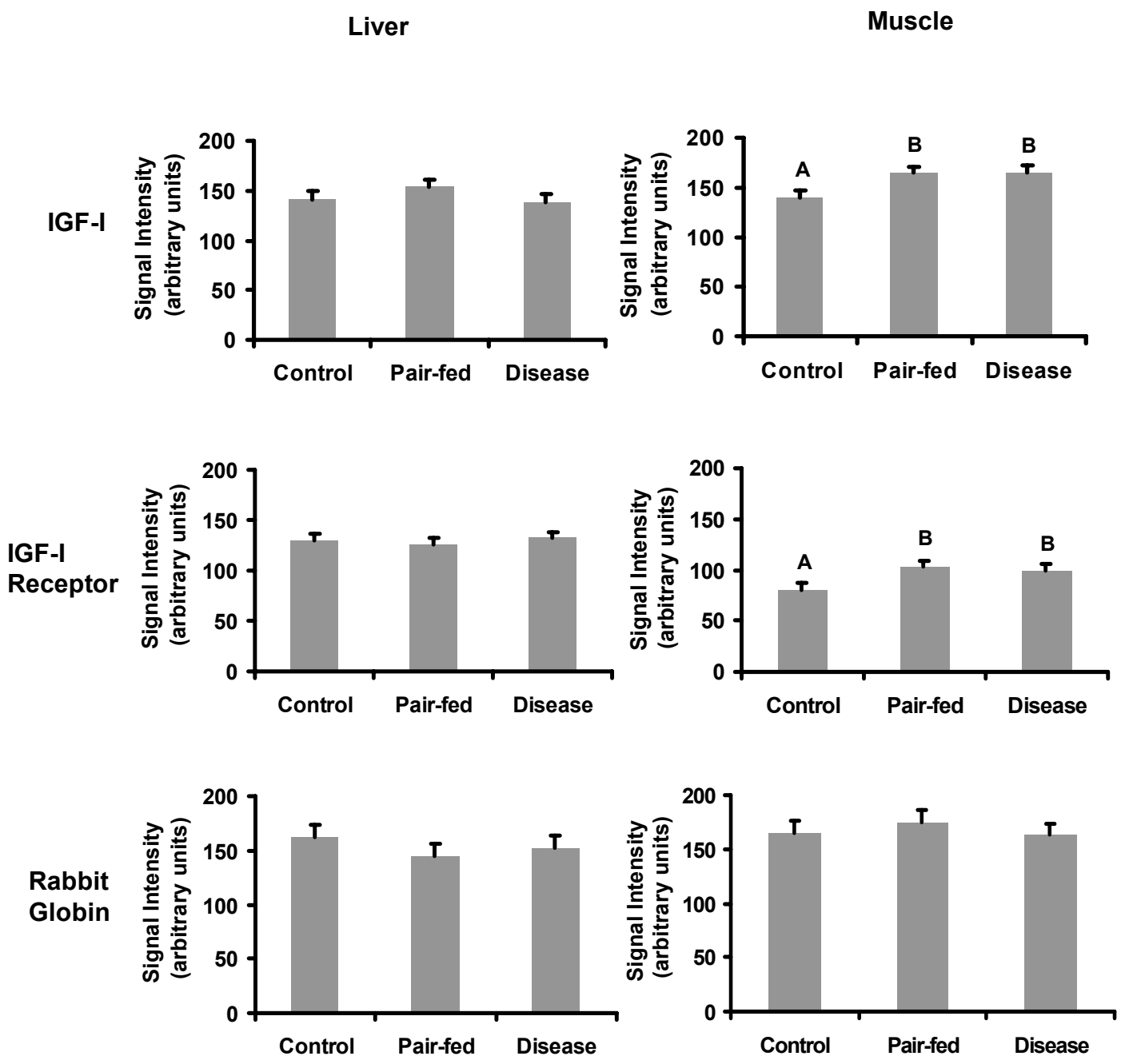

Figure 16. Expression of mRNA for IGF-I, the IGF type-I receptor, and exogenously added rabbit globin in liver and semitendinosus muscle. Values shown are least square means ± SEM. The expression of IGF-I and the IGF type-I receptor are increased in the semitendinosus muscle of disease challenged and pair-fed pigs ($P < .05$). n = 8 to 10.

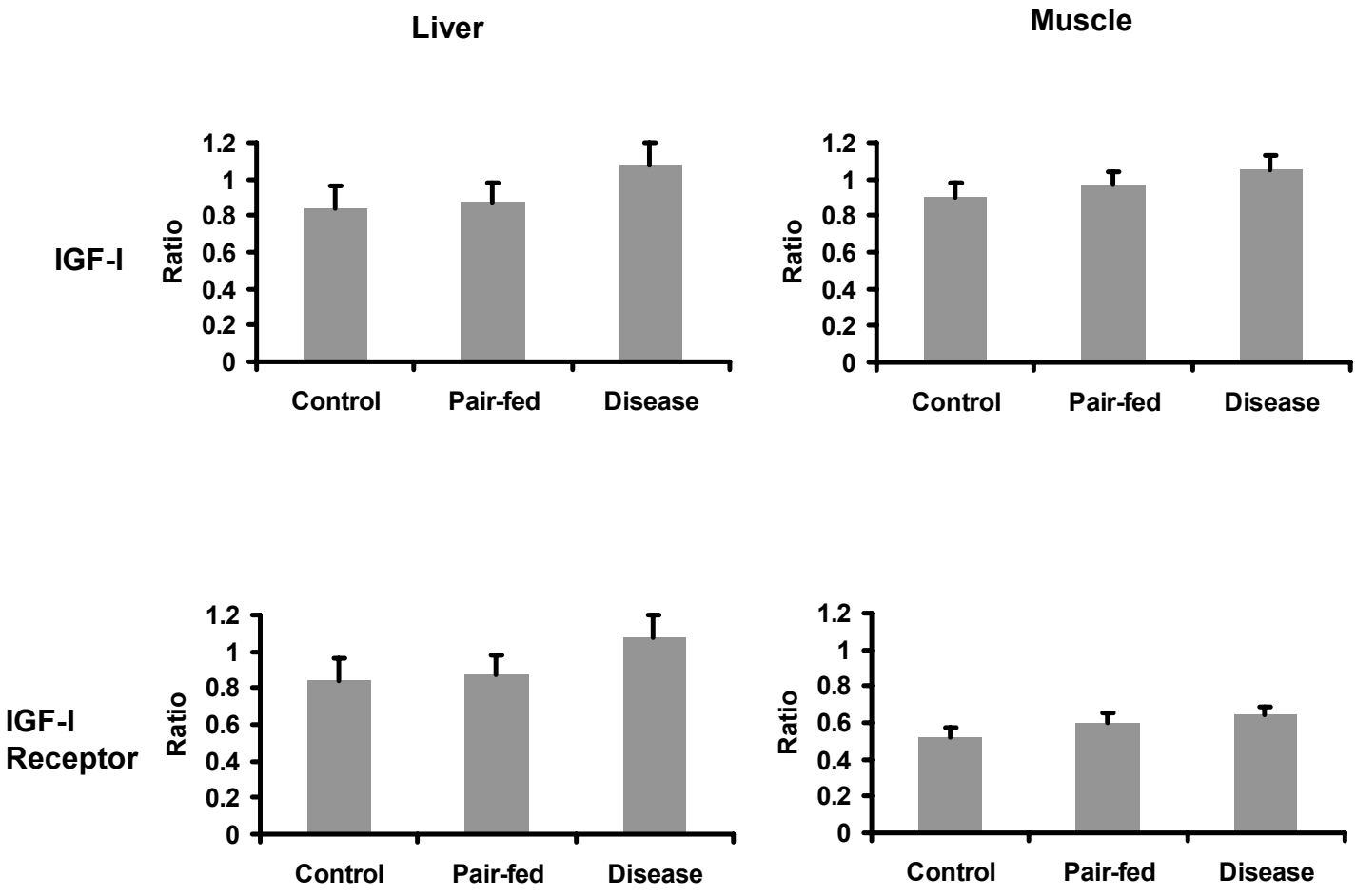

Figure 17. Ratio of the expression of mRNA for IGF-I and the IGF type-I receptor to the expression of exogenously added rabbit globin in liver and semitendinosus muscle. Values shown are least square means ± SEM. No differences were observed in the expression of IGF-I the IGF type-I receptor in liver and semitendinosus muscle, regardless of treatment ($P < .05$). n = 8 to 10.

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CHAPTER 5

DISSERTATION CONCLUSIONS

W. T. OLIVER

This Ph.D. program was conducted to examine the effects of the endogenous somatotropin (ST)/insulin-like growth factor (IGF) system and(or) administration of exogenous porcine ST (pST) on various commercially relevant applications of swine production. In chapter two, we examined the effect of an immunocastration vaccine (Improvac) and exogenous pST on growth performance and carcass characteristics in group-housed pigs in the finishing phase of production. We concluded that immunocastration, combined with daily injections of pST, drastically increased the performance of both boars and gilts above the levels observed when only Improvac or pST is administered. While pigs are routinely surgically castrated and pST is not currently available for commercial use in the United States, this research could have major impacts on swine production, in that both are proven technologies (individually and now in combination) should they become available for use in the future.

In chapter three we examined the effects of exogenous pST on pigs weaned from the sow at 10 d of age and fed either a high or low fat manufactured liquid diets. Previous research in humans had suggested that the source of dietary energy had an impact on the response to exogenous pST. We concluded that the ST-IGF axis was responsive to exogenous pST in the young pig, regardless of dietary energy source. Our results differed from some previous work, in that the young pig is not always responsive to pST treatment. We proposed that the level of feed intake and performance of pigs in the current experiment allowed us to realize the effects of pST. In hindsight, a group of pigs limit fed to achieve normal sow-reared growth rates would have been beneficial.

In chapter four we partially characterized the endogenous ST/IGF-I axis of

chronically health challenged pigs. We hypothesized that IGF-I would be downregulated, both systemically and at the tissue level, in pigs chronically infected with porcine reproductive and respiratory syndrome virus (PRRSv) and *M. hyopneumoniae (M. hyo*). We also hypothesized that pigs infected with PRRSv and *M. hyo* would have an attenuated response to pST. Circulating IGF-I, both basal and pST stimulated, did not differ between treatment groups. Also, disease challenge did not affect mRNA abundance of IGF-I or the type-I IGF receptor in liver or skeletal muscle. These data indicate that the ST/IGF system is unchanged in pigs infected with PRRSv and *M. hyo.* Furthermore, the ST/IGF system is responsive to exogenous pST, as indicated by the normal increase in IGF-I and decrease in PUN after pST treatment. One logical progression of this research is a longer-term pST administration to determine if exogenous pST increase growth performance similarly to control pigs. In addition, the mechanisms underlying the decrease in growth in chronically health challenged pigs has yet to be determined, particularly regarding the ST/IGF system. This research has determined that circulating IGF-I and liver and muscle IGF-I mRNA abundance are not implicated. However, this does not rule out the down regulation of the ST/IGF system in health-challenged pigs. The IGF binding proteins need to be characterized in serum and tissue, as well as the protein levels of IGF-I and the IGF type-I receptor in liver and musle. When these components have been examined, conclusions can be made as to whether or not the ST/IGF system is responsible for the reduction in growth of chronically health challenged pigs, at least to the level of the cell membrane.