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Dietetic supplementation of weaning piglets with caramel plant wastewater: growth and components of the insulin-like growth factor axis[1,2]

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ABSTRACT

An experiment was conducted to evaluate the expression of insulin-like growth factor-I (IGF-I) and IGF-binding proteins (IGFBPs -II and -III) in response to a 10% inclusion of caramel plant wastewater (CPWW) in weaning pig diets; the objective was to assess associations between those growth-related proteins to feed intake (FI) and body weight gain (BWG). Sixteen purebred Landrace piglets were randomly distributed among eight pens (a gilt and boar per pen) and assigned to one of two treatments: 0% (control) and 10% inclusion of CPWW. During four consecutive weeks, live weight and FI were recorded. Blood samples were drawn by jugular venipuncture during the first, second, and third weeks of the experiment and serum levels of IGF-I, IGFBP-II and IGFBP-III were determined. Feed intake, BWG and feed efficiency (FE) were not affected (P > 0.05) by the addition of 10% CPWW to

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the diet, nor was animal health status visibly affected. Serum IGF-I levels were higher in control animals ($P < 0.05$) and increased from d 14 to d 28 of the experimental period ($P < 0.05$). Weekly increases were observed for IGFBP-III ($P < 0.05$) whereas IGFBP-II circulating levels decreased from d 14 to d 28 of the post-weaning test period. Simple correlation analysis revealed that there was a positive association between circulating levels of IGF-I and IGFBP-III ($r = 0.88$; $P < 0.0001$). However, the opposite was observed between these two and IGFBP-II ($r = -0.84$, $P < 0.0001$; $r = -0.67$, $P < 0.0025$, respectively). The changes observed in circulating levels of IGF-I, IGFBP-III and IGFBP-II were associated with weekly increases in FI and BWG that occurred during the entire experimental period ($P < 0.05$).

Key words: insulin-like growth factor (IGF-I), IGF binding proteins, weight gain, feed intake

RESUMEN

Suplementación dietética con aguas residuales de una fábrica productora de caramelo y su efecto sobre el crecimiento de cerdos destetados y componentes del eje de factores de crecimiento semejantes a insulina

Se realizó un experimento para determinar si la inclusión de un 10% de aguas residuales de una fábrica de caramelo (CPWW, por sus siglas en inglés) en la dieta de cerdos post-destete resulta en cambios en los niveles del factor de crecimiento semejante a insulina-I (IGF-I, por sus siglas en inglés) y proteínas fijadoras de IGF (IGFBPs-II y -III, por sus siglas en inglés) en la sangre, y si éstos se asocian a diferencias en consumo de alimento (CA), ganancia en peso (GP), y eficiencia de conversión (EC). Dieciséis cerdos de raza Landrace se distribuyeron al azar entre ocho jaulas (una cerda y un cerdo por jaula) y se asignaron a uno de dos tratamientos: 0% (control) y 10% de inclusión de CPWW. Durante cuatro semanas consecutivas se registró el peso vivo y el consumo de alimento de los animales. Se recolectaron muestras de sangre a través de sangrado yugular durante la primera, segunda y tercera semana del experimento y se determinaron los niveles de IGF-I, IGFBP-II y IGFBP-III en el suero. No hubo efecto significativo de la adición de 10% CPWW en la dieta sobre CA, GP y EC ($P > 0.05$). Mediante apreciación visual se determinó que la inclusión de CPWW no tuvo efectos adversos en la salud de los animales. Los niveles de IGF-I fueron más altos para los animales control ($P < 0.05$) y aumentaron del día 14 al día 28 del periodo post-destete ($P < 0.05$). Se observó un aumento semanal en los niveles de IGFBP-III ($P < 0.05$), mientras que los niveles de IGFBP-II disminuyeron a partir del día 14 al día 28 ($P < 0.05$). El análisis de correlación simple reveló que existe una asociación positiva entre los niveles de IGF-I y IGFBP-III circulando en la sangre ($r = 0.88$; $P < 0.0001$). Sin embargo, se observó un efecto opuesto entre éstos y IGFBP-II ($r = -0.84$, $P < 0.0001$; $r = -0.67$, $P < 0.0025$, respectivamente). Los cambios observados en los niveles sanguíneos de IGF-I, IGFBP-III y IGFBP-II se asociaron a aumentos semanales en CA y GP que ocurrieron durante todo el periodo experimental ($P < 0.05$).

Palabras clave: factor de crecimiento semejante a insulina, proteínas fijadoras, ganancia en peso, consumo de alimento

INTRODUCTION

Swine producers have decreased the age at weaning in order to increase the number of pigs weaned per sow annually and thus improve profitability (Kerr et al., 1998). Early weaned piglets require highly

digestible diets, including high-quality protein ingredients, all of which makes diets more expensive (Richert et al., 1996). The acquisition of complex diets (for piglets under 21 days of age) represents a problem in Puerto Rico because there is no local production of this kind of diet and the ones available are too expensive to be attractive to swine producers. Therefore, there is a need to identify low-cost ingredients that can be added to diets without compromising animal performance and that can reduce production costs. Recent studies have used different percentages of wastewater from a caramel producing plant (CPWW) in the diets of weaning piglets (León et al., 2003; Jiménez, 2003). An improved growth performance was obtained with up to 7.5% CPWW in the diet (Jiménez, 2003).

The hormonal regulation of growth is multi-factorial and a group of proteins known to significantly affect animal growth and metabolism is the insulin-like growth factor (IGF) system (IGF-I, IGF-II, IGF cell surface receptors and IGF-binding proteins), which has been implicated as participating in many biological processes of relevance to livestock production systems. These processes include pre- and postnatal growth, lactation and reproduction, and immune function. The IGF system plays an important physiological role in the growth and development of mammals by acting locally in specific organs or systemically through circulating IGF-I (Werner et al., 1994). The actions of both IGF-I and IGF-II are mediated by specific insulin-like growth factor binding proteins (IGFBP). The IGFBP can act in an endocrine, paracrine, or autocrine fashion, and recent in vitro and in vivo findings suggest that IGFBP function independently of the IGF as growth modulators (Firth and Baxter, 2002). Even though tremendous progress has been achieved in meat production and processing technologies, a basic understanding of animal growth, as it relates to the physiology of IGFBP and their IGF independent effects, has the potential for improving meat production efficiency. The hypothesis to be tested in this experiment was that a 10% dietary inclusion of CPWW might induce indirect changes in relative levels of IGFBP and exert a positive effect on growth. If so, CPWW inclusion would constitute an excellent alternative for disposal of the wastewater by-product and also benefit meat production.

MATERIALS AND METHODS

Sixteen Landrace shoats from two different litters, averaging 21 ± 2 days of age, and 6 kg of body weight (BW), were distributed among eight pens (one of each gender per pen) at the Department of Animal Industry Swine Farm located in Lajas, Puerto Rico. For four consecutive weeks the piglets were fed daily a commercial weaning diet, at a

rate of 8% of their BW, supplemented with either 0 or 10% of CPWW. This by-product was obtained from a Coca-Cola Company plant located in Gurabo, Puerto Rico, and was mixed with the commercial diet. The animals were weighed weekly and observed periodically for the presence of diarrhea or any other visible change in their health status. Blood was drawn once a week by jugular venipuncture and sample pools were subjected to Radioimmunoassay (RIA) to detect IGFBP-II, and Immunoradiometric Assay (IRMA) to detect IGF-I and IGFBP-III (Diagnostic Systems Laboratories, Webster, Texas).[8] A diagram of the pooling strategy is provided below:

Days	0	14	28
Week Effect: C + T = ♀ + ♂	1	1	1
Sex by Treatment Effect: ♀ ♂ C T C T	4	4	4
Treatment Effect: C (all) T (all)	2	2	2

C = Control Pigs
T = Pigs supplemented with a 10% CPWW
♀ = Gilt
♂ = Boar
Total Number of Pools = 21

The variables feed intake (FI) and body weight gain (BWG) and feed efficiency (FE) were analyzed as a completely randomized design using a lineal model (SAS, 1996) with the initial bodyweight per pen as covariable. A factorial experimental design was used to study the effect of treatments (0 and 10% CPWW), sex (male and female), week (day 0, 14, 28 of blood collection) and the interaction between sex and treatment with respect to circulating levels of IGF-I, IGFBP-II and IGFBP-III (GLM Procedure; SAS, 1996). The correspondent means were separated by using the Bonferroni test, and the degree of association between IGF-I, IGFBP-II and IGFBP-III and the performance traits evaluated was established by Pearson Correlation Coefficients (Corr Procedure; SAS, 1996). All statements of significance were based on a probability level of less than 0.05 unless otherwise stated.

[8]Company names in this publication are used only to provide specific information. Mention of a name does not constitute a warranty by the Agricultural Experiment Station of the University of Puerto Rico, nor is this mention a statement of preference over other companies.

RESULTS AND DISCUSSION

The dietary addition of 10% of CPWW did not significantly affect productive performance in this trial (Figures 1 and 2). Feed intake and BWG were similar in the control (0% of CPWW) and the supplemented group (10% of CPWW) (Figure 2). The control group showed an advantage in FE by a margin of 0.08 kg weight gain/kg of FI (Figure 2), but this difference was not significant. These results differ from those obtained by Jiménez (2003), in which the CPWW-supplemented pigs showed greater FI, BWG and FE than the controls. However, the levels of CPWW used in that study were lower than the level evaluated in the present trial (up to 7.5% vs. 10%, respectively). Neither diarrhea nor any other health problems were observed in the pigs supplemented with 10% of CPWW. Weekly increases in FI and BWG were observed during the experiment (Figure 3).

Serum IGF-I levels were significantly higher in control animals (99 ng/ml) than in those supplemented with CPWW (62 ng/ml), as determined from pooled samples collected at days 0, 14 and 28 of the experiment. Serum IGF-I levels significantly increased from day 14 to day 28 of the experiment (Table 1). Weekly increases were observed for IGFBP-III, whereas IGFBP-II circulating levels decreased from day 14 to day 28 of the post-weaning test period (Table 1). Simple correlation analysis revealed that there was a positive association between circulating levels of IGF-I and IGFBP-III ($r = 0.88$; $P < 0.0001$). However, the opposite was observed between each of these and IGFBP-II ($r = -0.84$, $P < 0.0001$; $r = -0.67$, $P < 0.0025$, respectively). Unlike growth hormone,

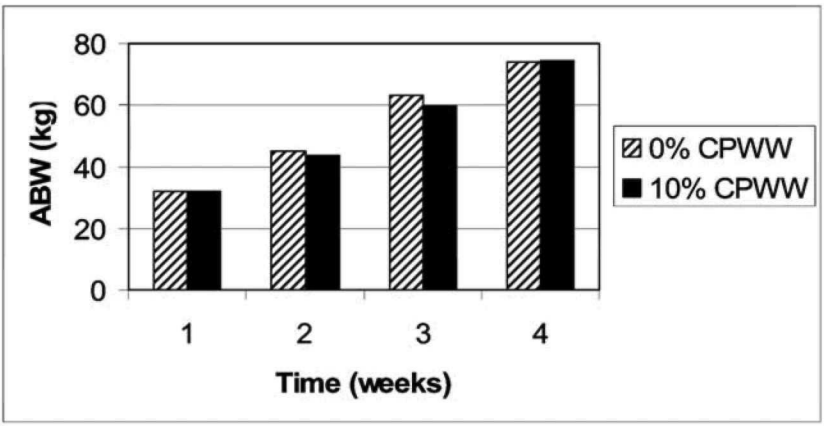

FIGURE 1. Weekly average body weight (ABW) of control pigs (0% CPWW) and pigs supplemented by a 10% inclusion of CPWW during the postweaning period.

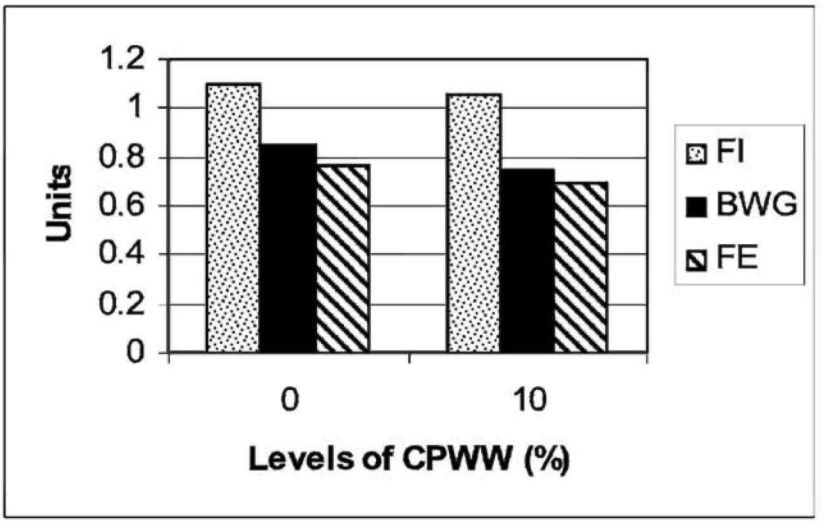

FIGURE 2. Daily feed intake (FI; kg), body weight gain (BWG; kg) and feed efficiency (FE) during the 4-wk experimental period as affected by dietary supplementation with CPWW.

which is secreted in a pulsatile manner, IGF-I levels remain fairly constant throughout the day (Bach, 1999) and can be reliably determined from a single blood sample (Bishop et al., 1989). IGF-I serum concentration is associated with growth traits in many livestock species (Anderson et al., 1988; Graml et al., 1994) and may be useful as a physiological indicator in selection programs designed to improve weight and growth rate in pigs (Buonomo et al., 1987). IGF-I mediates many of the growth-promoting effects of growth hormone and regulates postnatal growth and development. However, the actions of the IGF-I and -II (IGFs) are modulated by a family of six high affinity-binding proteins (IGFBP-I-VI) that have been identified by molecular cloning of their cDNAs from rat and human tissues (Shimasaki and Ling, 1991). These proteins bind IGFs with an affinity equal to or greater than that of the IGF-I receptor (Rechler and Clemmons, 1998) and they can act in an endocrine, paracrine, or autocrine fashion. IGFBP-II and -III, like IGF-I, are also responsive to growth hormone (Cohick et al., 1992; Harrell et al., 1999) and may be useful indicators of the rate of gain and carcass composition in livestock. IGFBP-II is a distinct protein whose plasma concentration is inversely related to growth hormone (Binoux et al., 1986), whereas IGFBP-III is a glycoprotein whose plasma concentration is directly related to growth hormone secretory status (Baxter and Martin, 1986). We found

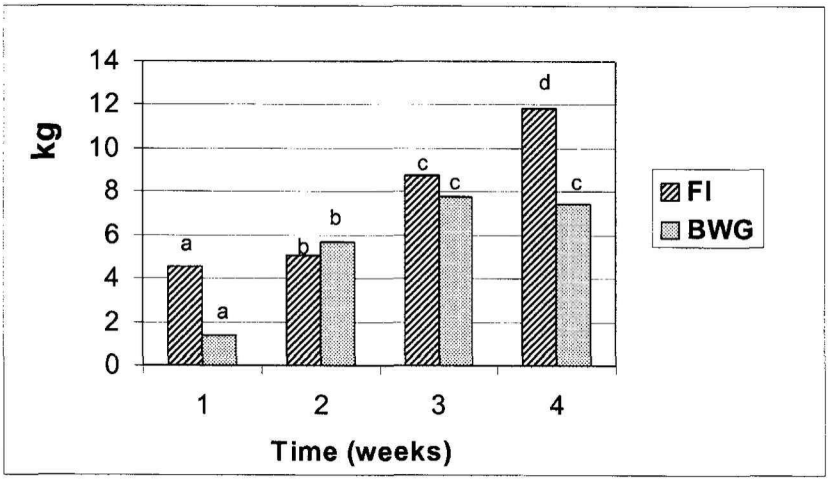

FIGURE 3. Feed intake (FI) and body weight gain (BWG) per week of post-weaning pigs. Within each variable, means lacking a common superscript letter differ ($P < 0.05$).

that circulating levels of IGFBP-II were higher than the levels of both IGF-I and IGFBP-III, respectively, even though they decreased from day 14 to day 28 of the experimental period (Table 1). Generally, IGFBP-III is the most abundant IGFBP species in serum and milk. It is produced mainly by non-parenchymal hepatic cells and circulates in serum, binding IGF-I or IGF-II in conjunction with an acid-labile subunit (ALS) to form a 150-kDa circulating (ternary) complex at a serum concentration

TABLE 1.—*Serum IGF-I, IGFBP-II and IGFBP-III levels of post-weaning pigs.*[1,2]

	Days		
Proteins (ng/ml)	0	14	28
IGF-I[3]	14.0[a]	52.8[a]	174.7[b]
IGFBP-III[4]	122.8[a]	201.5[b]	252.0[c]
IGFBP-II[5]	1035.0[a]	1018.5[a]	625.0[b]

[1]Samples collected at day 0, 14, and 28 of the experimental period were individually pooled.

[2]Between columns means lacking a common superscript differ ($P < 0.05$).

[3]IGF-I = Insulin-like growth factor-I levels determined by Immunoradiometric assay (Diagnostic Systems Laboratories, Inc.).

[4]IGFBP-III = Insulin-like growth factor binding protein-III levels determined by Immunoradiometric assay (Diagnostic Systems Laboratories, Inc.).

[5]IGFBP-II = Insulin-like growth factor binding protein-II levels determined by radioimmunoassay (Diagnostic Systems Laboratories, Inc.).

of about 100 nM, which increased the IGF-I half life to 15 h (Rechler and Clemmons, 1998). This is in accordance with the strong positive correlation found between these two proteins in the present study (Table 1).

The precise roles of individual IGFBP are still unknown, mainly because of the great complexity of their actions and regulation, but also because the overwhelming majority of the available information about the IGFBP is derived from in vitro studies. IGFBP-II appears to play a key role in myogenesis (Ernst et al., 1992; Ernst et al., 1996; Fligger et al., 1998; Gerrard et al., 1999). The level of expression of IGFBP-II mRNA and protein was found to be higher in proliferating turkey myogenic satellite cells (Ernst et al., 1996) and mouse myoblasts (Ernst et al., 1992) and to decrease gradually as differentiation progresses. This finding has been associated with a sequestration of IGF-I by IGFBP-II, which makes that growth factor less available to the myogenic satellite cells (Fligger et al., 1998). Schneider et al. (2000) reported that the most prominent phenotype in IGFBP-II transgenic mice was one of a reduced somatic growth and that overexpression of IGFBP-III under a ubiquitous promoter resulted in selective organomegaly. In our study, circulating levels of both IGFBP-II and IGFBP-III were much higher than the levels of IGF-I. Therefore, it appears that these two binding proteins are somehow regulating the actions of IGF-I (Table 2) and also probably functioning in an IGF-I independent manner (according to serum levels; Table 1). The changes observed in circulating levels of IGF-I, IGFBP-II and IGFBP-III might in turn have regulated the weekly increases in FI and BWG that occurred during the 5-wk experimental period. In that respect, a strong positive correlation was observed be-

TABLE 2.—*Pearson correlation coefficients and associated P-values for Serum IGF-I[1], IGFBP-III[2], IGFBP-II[3], feed intake (FI) and body weight gain (BWG).*

Trait	IGF-I	IGFBP-III	IGFBP-II	FI	BWG
Week	0.88	0.92	-0.64	0.89	0.97
	0.0001	0.0001	0.0042	0.0001	0.0001
IGF-I		0.88	-0.84	0.90	0.91
		0.0001	0.0001	0.0001	0.0001
IGFBP-III			-0.67	0.74	0.86
			0.0025	0.0004	0.001
IGFBP-II				-0.72	-0.69
				0.0008	0.0014
FI					0.97
					0.0001

[1]IGF-I = Insulin-like growth factor-I
[2]IGFBP-III = Insulin-like growth factor binding protein-III
[3]IGFBP-II = Insulin-like growth factor binding protein-II

tween IGF-I, IGFBP-III, FI, and BWG (Table 2). The opposite was observed for IGFBP-II. These observations provide further evidence of the role played by components of the IGF-I axis in economically important traits related to food animal production.

In this study no significant effects on the performance of weaning pigs resulted from dietary supplementation with 10% of waste CPWW. Feed intake, BWG and FE were not statistically different from those of the control. However, changes in circulating levels of IGF-I, IGFBP-III, and IGFBP-II observed in control pigs and pigs supplemented with a 10% inclusion of CPWW were associated with weekly increases in FI and BWG that occurred during a 28-d post-weaning experimental period, all of which reaffirms that these proteins play a key role in swine growth regulation.

LITERATURE CITED

Anderson, P. T., W. G. Bergen, R. A. Merkel, W. J. Enright, S. A. Zinn, K. R. Refsal and D. R. Hawkins, 1988. The relationship between composition of gain and circulating hormones in growing beef bulls fed three dietary crude protein levels. *J. Anim. Sci.* 66:3059-3067.

Bach, L. A., 1999. The insulin-like growth factor system: basic and clinical aspects. *Aust. NZ. J. Med.* 29:355-361.

Baxter, R. C. and J. L. Martin, 1986. Radioimmunoassay of growth hormone dependent insulin-like growth factor binding protein in human plasma. *J. Clin. Invest.* 78:1504-1512.

Binoux, M., P. Hossenlopp, S. Hardouin, D. Seurin, C. Lassarre and M. Gourmelen, 1986. Somatomedin (insulin-like growth factor)-binding proteins: Molecular forms and regulation. *Horm. Res.* 24:141-151.

Bishop, M. D., R. C. M. Simmen, F. A. Simmen and M. E. Davis, 1989. The relationship of insulin-like growth factor-I with postweaning performance in Angus beef cattle. *J. Anim. Sci.* 67:2872-2880.

Buonomo, F. C., T. J. Lauterio, C. A. Baile and D. R. Campion, 1987. Determination of insulin-like growth factor 1 (IGF1) and IGF binding protein levels in swine. *Dom. Anim. Endocrinol.* 4:23-31.

Cohick, W. S., M. A. McGuire, D. R. Clemmons and D. E. Bauman, 1992. Regulation of insulin-like growth factor-binding proteins in serum and lymph of lactating cows by somatrotopin. *Endocrinol.* 130:1508-1514.

Ernst, C. W., R. H. McCusker and M. E. White, 1992. Gene expression and secretion of insulin-like growth factor-binding proteins during myoblast differentiation. *Endocrinology* 130:607-615.

Ernst, C. W., D. C. McFarland and M. E. White, 1996. Expression of insulin-like growth factor II (IGF-II), IGF binding protein-2 and myogenin during differentiation of myogenic satellite cells derived from the turkey. *Differentiation* 61:25-33.

Firth, S. M. and R. C. Baxter, 2002. Cellular actions of the insulin-like growth factor binding proteins. *Endocr. Rev.* 23: 824-854.

Fligger, J. M., P. V. Malven, M. E. Doumit, R. A. Merkel and A. L. Grant, 1998. Increases in insulin-like growth factor binding protein-2 accompany decreases in proliferation and differentiation when porcine muscle satellite cells undergo multiple passages. *J. Anim. Sci.* 76:2086-2093.

Gerrard, D. E., C. S. Okamura and A. L. Grant, 1999. Expression and location of IGF binding proteins-2, -4 and -5 in developing fetal tissues. *J. Anim. Sci.* 77:1431-1441.

Graml, R., A. Olbrich-Bludau, M. Schwab, E. Schallenberger, D. Schams and F. Pirchner, 1994. Relationship between plasma hormone and metabolite levels and breeding values of bulls. *J. Breed. Genet.* 112:313-326.

Harrell, R. J., M. J. Thomas, R. D. Boyd, S. M. Czerwinski, N. C. Steele and D. E. Bauman, 1999. Ontogenic maturation of the samototropin/insulin-like growth factor axis. *J. Anim. Sci.* 77:2934-2941.

Jiménez, E., 2003. Use of waste waters from a caramel producing plant as a supplement in the diet of early weaning and finishing pigs. M.S. Thesis, Department of Animal Industry, University of Puerto Rico, Mayagüez Campus. 57 pp.

Kerr, C., R. D. Goodband, J. W. Smith, R. E. Musser, J. R. Bergstrom, W. B. Nessmith, M.D. Tokach and J. L. Nelssen, 1998. Evaluation of potato proteins on the growth performance of early-weaned Pigs. *J. Anim. Sci.* 76: 3024-3033.

León, F. J., R. Sánchez, C. S. Santana and A. Rodríguez, 2003. Waste water from a caramel industry as a partial substitute in diets for pigs. *J. Agric. Univ. P.R.* 87: 87-90.

Rechler, M. M. and D. R. Clemmons, 1998. Regulatory actions of insulin-like growth factor-binding proteins. *Trends Endocrinol. Metab.* 9:176-183.

Richert B. T., K. R. Cera, A. P. Schinckel, L. K. Clark and J. J. Turek, 1996. Effect of nursery diet complexity and weaning age on pig growth performance and health status. Purdue Swine Day. 1-4.

SAS, 1996. The SAS System for Windows (Version 6.12, TS020). SAS Institute, Inc., Cary, NC.

Schneider, M. R., H. Lahm, M. Wu, A. Hoeflich and E. Wolf, 2000. Transgenic mouse models for studying the functions of insulin-like growth factor-binding proteins. *FASEB J.* 14:629-640.

Shimasaki, S. and N. Ling, 1991. Identification and molecular characterization of insulin-like growth factor binding proteins (IGFBP-1, -2, -3, -4, -5, and -6). *Prog. Growth Factor Res.* 3:243-266.

Werner, H., M. Adamo, C.T. Roberts, Jr. and D. LeRoith, 1994. Molecular and cellular aspects of insulin-like growth factor action. *Vitam. Horm.* 48:1-58.