ELSEVIER

PII: S0308-8146(97)00010-1

Food Chemistry, Vol. 59, No. 4, pp. 499–504, 1997
Published by Elsevier Science Ltd
Printed in Great Britain
0308-8146/97 $17.00+0.00

Biotechnology for porcine products and its effect on meat products

M. B. Solomon,[ab] V. G. Pursel,[ac] R. G. Campbell[d] & N. C. Steele[ae]

[a]*Beltsville Agricultural Research Center, Agricultural Research Service, USDA, Livestock and Poultry Sciences Institute, Beltsville, Maryland, USA*
[b]*Meat Science Research Laboratory, Beltsville, Maryland, USA*
[c]*Gene Evaluation and Mapping Laboratory, Beltsville, Maryland, USA*
[d]*Bunge Meats Ltd., Corowa, NSW 2626, Australia*
[e]*Growth Biology Laboratory, Beltsville, Maryland 20705-2350, USA*

(Received 15 March 1996; revised version received 5 July 1996; accepted 5 July 1996)

Recombinant DNA technology has provided a mechanism for large scale production of somatotropin (growth hormone). There is no question that exogenous administration of somatotropin (ST) to pigs significantly improves efficiency of growth and carcass composition. Microinjecting foreign DNA into the pronucleus of fertilized ova is the predominant method employed to produce transgenic animals. The goal of producing transgenic pigs is to improve productive traits and carcass composition, enhance animal health and produce useful human health products. With greater emphasis on lean tissue accretion and less lipid deposition, either exogenous administration of ST or transgene technology can be used as a tool to maximize genetic potential for protein accretion and/or lipid depletion. The magnitudes of response for protein accretion and lipid depletion differ between these two biotechnological strategies. However, both offer means for progress in meeting consumer demands for lean meat. Published by Elsevier Science Ltd

INTRODUCTION

Biotechnology is the application of molecular biology techniques to problems and questions in many diverse disciplines. The use of science for the improvement of muscle foods has involved natural selection of dominant traits, selection of preferred traits by crossbreeding, the use of endogenous and exogenous growth factors and, ultimately, gene manipulation to produce desirable changes in meat/carcass quality and yield. Until recently, improvements in the quality of meat products that reached the market place were largely the result of post-harvest technology. Extensive post-harvest efforts have been implemented to improve or to control the tenderness, flavor and juiciness which are the sensory attributes that make meat products palatable.

A wide range of biotechnology strategies for altering the balance between lean and adipose tissue growth and deposition in meat-producing animals are available. These include genetic selection and management (production) strategies. More recently, the confirmation of the growth-promoting and nutrient repartitioning effects of somatotropin, somatomedin, β-adrenergic agonists, immunization of animals against target circulating hormones or releasing factors and gene manipulation techniques have given rise to a technological revolution for altering growth and development in meat producing animals. This paper will present results of research efforts involving both exogenous administration of porcine somatotropin and genetic engineering of swine from the US Department of Agriculture, Agricultural Research Service, Beltsville laboratories.

Exogenous porcine somatotropin administration

There is a growing database supporting the use of pituitary or recombinantly derived porcine somatotropin (pST) as an agent to improve efficiency of growth and carcass composition in swine. Turman and Andrews (1955) and Machlin (1972) were the first to demonstrate that daily exogenous administration (injection) of highly purified pST dramatically altered nutrient use resulting in improved growth rate and feed conversion of growing-finishing pigs. Pigs injected with pST had less (35%) fat and more (8%) protein.

However, their original observations were of little practical significance because purification of porcine ST from pituitary glands was not economical. A single dose

of purified porcine ST required 25–100 pituitary glands. More recently, the development of recombinant deoxyribonucleic acid (DNA) technology has provided a mechanism for the large scale production of somatotropin. There is a growing database supporting the use of recombinantly derived pST (rpST). No significant differences in the effectiveness of pST vs rpST have been observed.

With greater emphasis on lean tissue deposition and less lipid, the optimal genetic potential for protein deposition in an animal is a very important concept in that this potential, or ceiling, defines the dietary protein requirement of the animal. In defining the optimal genetic potential for protein deposition, ST is used as a tool to maximize the utilization of the genetic potential for protein accretion. Administration of pST to growing pigs elicits a pleiotropic response that results in altered nutrient partitioning. In studies with growing pigs, significant improvements of 40% in average daily gain and 30% in feed conversions can be achieved by administration of pST. Research has also shown that the effect of pST is enhanced by good management and nutritional practices (Campbell *et al.*, 1990; Caperna *et al.*, 1990).

Carcass composition.

Lipid composition studies have demonstrated that the lipid content of pST-treated pig carcasses was significantly less than in controls. For comparison purposes, in this paper pST administration is compared to transgenic technology. Both the control and treated pigs (Duroc x Yorkshire intact males, $n = 12$) were fed a diet containing 3.5 Mcal digestible energy/kg and pST pigs were injected daily with exogenous pST (100 $\mu gkg^{-1}d^{-1}$) for 31 days starting at 60 kg live weight with no withdrawal period. All pigs were slaughtered at approximately 100 kg live weight. The left side of each intact carcass was ground and tissue samples were analyzed for lipid composition and cholesterol content (details for analytical methods see Solomon *et al.*, 1994*b*). Total carcass fat was reduced 26% in pST-treated pigs (Table 1) compared to controls. Analysis of fatty acids (Fig. 1) showed that carcasses from pST-treated pigs contained less (22%) saturated fatty acids (SFA), less (31%) monounsaturated fatty acids (MUFA) and similar levels of polyunsaturated fatty acids (PUFA) than controls. Cholesterol content of ground carcass tissue was not different between pST treated pigs and controls.

Lean tissue composition

Loin eye area, an indicator of carcass muscling (lean tissue accretion via hypertrophy), increased in size (28%) as a result of pST treatment (Table 1). The administration of pST resulted in lean (*longissimus* muscle) tissue containing 27% less intramuscular fat (Table 1) than controls and as much as 40% less saturated fatty acids (SFA), 37% less monounsaturated fatty acids (MUFA) and no difference in polyunsaturated fatty acids (PUFA) compared to controls (Fig. 2). The decrease in total SFA and MUFA, and virtual no change in PUFA in both the carcass and lean tissue, support the conclusion that the mechanism by which pST decreases carcass fat content in pigs is by the inhibition of lipogenesis (Etherton *et al.*, 1995). A decrease in fat synthesis would lead to a decrease in the production of SFA and MUFA with little effect (change) in the amount of PUFA. The majority of PUFA in pig tissues are the result of dietary fatty acids linoleic and linolenic, and are not synthesized. However, the possibility of an increased turnover of storage lipids, at the level of triacylglycerol synthesis or hydrolysis, exists (Clark *et al.*, 1992). Cholesterol content of lean tissue from pigs receiving pST was 11% greater than controls (Table 1).

Meat tenderness

Administration of pST represents a technology with a promise for packers and retailers to offer leaner pork products. The administration of pST to castrated males (barrows) has been shown to reduce meat tenderness by as

Table 1. Comparison[a] of total carcass and lean tissue lipid and cholesterol content, *longissimus* muscle area and shear force for transgenic pigs and pigs administered porcine somatotropin

Item N	T-control[b] 17	T-bGH 7	T-oGH 5	T-hGH 5	pST 6	pST-control 6	SEM
Carcass							
Total lipid, g $100g^{-1}$	27.00[c]	4.49[e]	4.82[e]	9.85[e]	18.64[d]	25.18[c]	1.7
Cholesterol, mg/$100g^{-1}$	68.71	77.18	67.87	69.85	68.48	70.72	2.5
Lean							
Total lipid, g $100g^{-1}$	2.89[c]	1.38[d]	0.96[d]	1.16[d]	2.33[c]	3.21[c]	0.5
Cholesterol, mg $100g^{-1}$	48.64	55.58	49.33	50.75	50.13	45.38	1.5
Longissimus muscle area, cm^2	33.91[d]	32.37[d]	—	—	42.61[c]	33.29[d]	3.1
Shear-force, kg 1.27 cm^{-1}	3.32[e]	3.46[e]	—	—	4.83[d]	5.61[c]	0.5

[a]Wet weight basis.
[b]T-control = transgenic control.
[c,d,e]Means in a row with different letters are different ($p < 0.05$).

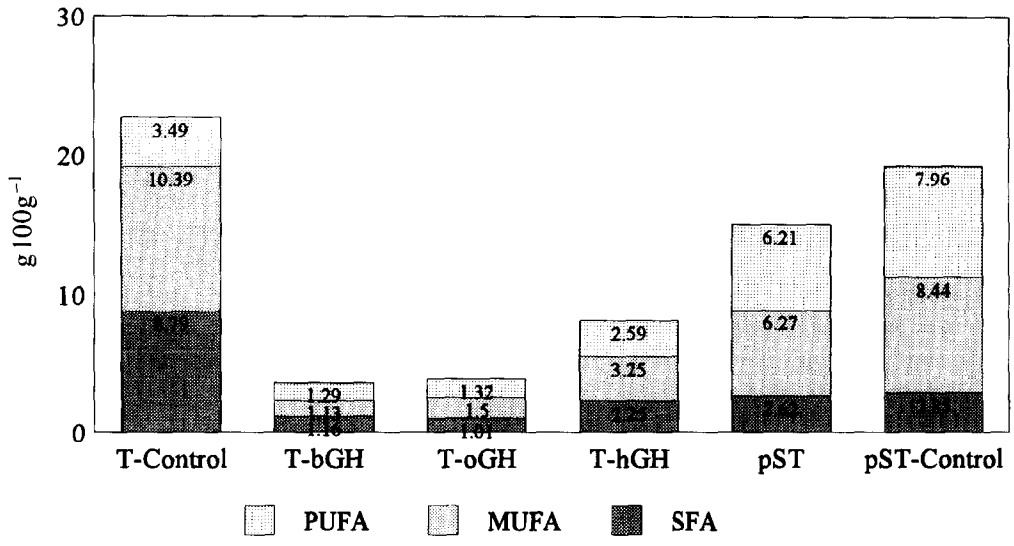

Fig. 1. Relative fatty cid composition for carcasses from transgenic and pST administered pigs.

much as 39% when compared to controls (Solomon *et al.*, 1990). On the contrary, in the intact male pST reduced shear-force tenderness (Table 1) by as much as 14% (5.61 vs. 4.83 kg). For details on shear-force evaluation procedures see Solomon *et al.*, (1994*a*). It is difficult to determine whether tenderness differences will be perceived by the consumer; however, it should be noted that most of the shear-force values were within those associated with normal pork products. In a recent study by Solomon *et al.* (1994*a*), time post-mortem of sampling muscle from pST-treated pigs for subsequent shear-force analysis had a significant effect on tenderness. Differences in shear-force tenderness between pST and control pigs (barrows) were virtually eliminated when loin chops were removed from the carcass and frozen within 1.5 h *post mortem* compared to controls (frozen 5 days *post mortem*). Some of the inconsistencies reported in the literature for shear-force and tenderness as a result of pST administration may be a result of inconsistencies in the time that the meat sample is removed and the time that the sample is frozen as well as the gender of the animal being treated.

Minimal observable differences in processing properties, yields, color retention or composition of products from control and pST-treated pigs have been observed (McKeith, 1993). From the wealth of literature, it appears that pST affects carcass composition and not quality (other than possibly tenderness). However, pale, soft, exudative muscle (PSE) has been observed in two of pST studies (Solomon *et al.*, 1990, 1991).

Transgenic pigs

In the past decade, development of recombinant DNA technology has enabled scientists to isolate single genes, analyze and modify their nucleotide structures, make

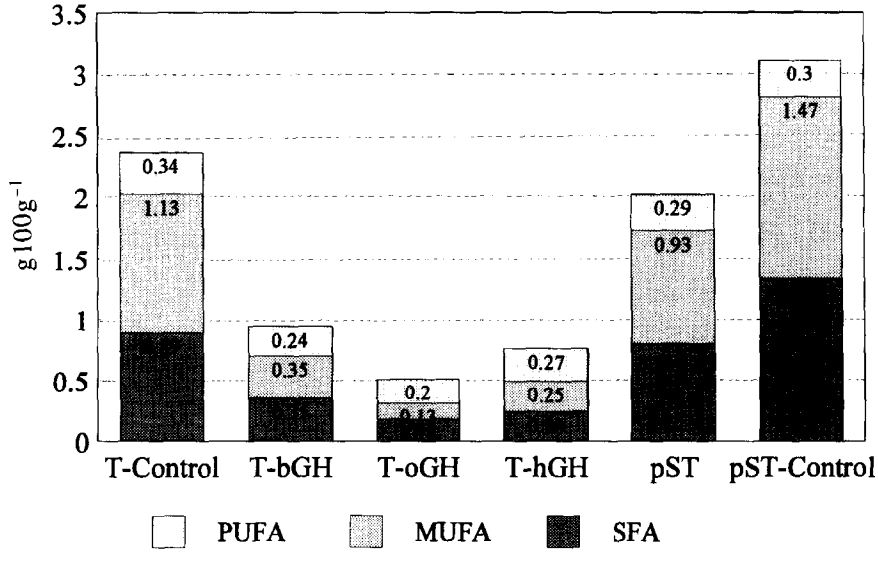

Fig. 2. Relative fatty cid composition for lean from transgenic and pST administered pigs.

copies of these isolated genes, and transfer copies into the genomes of livestock species. Such direct manipulation of genetic composition is referred to as 'genetic engineering' and the term 'transgenic animal' denotes an animal whose genome has been altered by the introduction of a 'foreign' gene. Excellent reviews by Wall *et al.* (1992) and Pursel *et al.* (1996) describe the techniques involved in genetic engineering of livestock.

The dramatic achievements in molecular biology during the past decade and the development of micromanipulation for early-stage embryos provided the combined capabilities for introducing cloned genes into the mouse genome in the 1980s (Brinster *et al.*, 1985; Palmiter & Brinster, 1986). The transfer of genes was immediately recognized as an important scientific achievement. Furthermore, the subsequent creation of the 'super' mouse by the transfer of a rat growth hormone (GH) gene provided the convincing evidence that demonstrated the potential offered by gene transfer (Palmiter *et al.*, 1982).

A number of transgenic swine have been engineered to contain various GH transgenes (Pursel & Rexroad, 1993). Marketing of pigs with elevated GH offers considerable potential value to both the producer and consumer. Even if only improved efficiency in feed utilization (as much as 20% reduction in feed consumption) is considered (Pursel *et al.*, 1996), the potential savings would amount to more than one billion dollars annually in lower feed costs if applied across the market hog population in the U.S. In addition, the producer would have the possibility of marketing 10 to 15% more pigs with the same fixed assets, because the genetically engineered pigs grow faster. In turn, consumers would benefit from having pork available with less fat at a lower cost than at present, and the environment would benefit from a 15% reduction in the quantity of waste being generated.

The public has expressed concern regarding the potential effect bioengineered genes might have on the consumer if the transgenically altered products are used for human food, particularly when the gene product is a hormone or bioactive peptide. Although biotechnology can increase agricultural productivity, produce greater profits, lower food costs, and improve the competitiveness in world markets, there are real and perceived adverse socioeconomic effects. This paper will not discuss these socioeconomic effects or acceptance.

Production of excess GH in the transgenic pigs caused multiple physiological effects, but did not result in 'giantism' as was expected based on the earlier production of 'super' mice as described by Palmiter *et al.* (1982). One unresolved major problem is the inability to design new genes that behave precisely as desired in all transgenic animals produced. Because our understanding of gene control is still limited, the new genes function correctly in only a small proportion of the transgenic animals generated. Furthermore, only 4% of transgenic embryos transferred into host mothers result in the birth of transgenic animals.

Transgenic pigs with excess GH levels exhibited numerous unique carcass traits (Solomon *et al.*, 1994*b*), some of which will be described in this paper. Reduced carcass fat, alteration of muscle fibers, thickening of the skin, enlargement of bones, and redistribution of major carcass components occurred in transgenic pigs. Some of these effects are similar to those observed after daily injection of pST, while others are considerably different. Possibly, these differences are the consequence of continual presence of excess GH in the transgenics while injections of pST provide a daily pulse of excess GH.

Carcass composition

A total of 17 transgenic pigs were compared to 17 sibling control pigs. All pigs (Duroc x Yorkshire ancestry) were fed a common diet and were slaughtered at an average live weight of 100 kg. The left side of each intact carcass was ground and tissue samples were analyzed for lipid composition and cholesterol content. Carcass fat (Table 1) was significantly reduced in transgenic pigs that expressed either a bovine (84%), ovine (82%) or human (64%) GH transgene compared to sibling controls. These dramatic reductions in carcass fat were not the result of inanition, as evident from enhanced rates of gain, but rather an interference in insulin's ability to stimulate lipogenesis (Pursel *et al.*, 1996), even though insulin was 20-fold higher in the transgenic pigs than in the sibling controls.

Analysis of fatty acids showed that carcasses from T-bGH, T-oGH and T-hGH pigs consistently contained less saturated fatty acids (SFA) (87, 85, and 70%, respectively) than sibling control pigs (Fig. 1). These differences in SFA were primarily a result of reductions in palmitic (C16:0), stearic (C18:0), and myristic (C14:0) acids. Palmitic and stearic acids accounted for 62 and 33%, respectively, of the total detectable SFA in the control group compared to 66 and 30%, respectively in the transgenic groups. Both myristic and palmitic acids have been reported to be hyperlipidemic and hypercholesterolemic (Keys *et al.*, 1965). Human consumption of hypercholesterolemic fatty acids have come under attack by health professionals. Bonamone and Grundy (1988) reported that a diet high in stearic acid did not elevate plasma levels of low density lipoprotein cholesterol. Carcasses from T-bGH, T-oGH and T-hGH pigs contained less total monounsaturated fatty acids (MUFA) (89, 86, and 69%, respectively) than sibling control pigs. Oleic acid was the major contribution of MUFA, 93% in control-pigs and 92% in all transgenic-pigs. Oleic acid is not considered to be an undesirable dietary fatty acid since it has the ability to reduce LDL cholesterol (Mattson & Grundy, 1985). Polyunsaturated fatty acids (PUFA) were reduced 63, 71, and 36% in T-bGH, T-oGH, and T-hGH, respectively, compared to controls. Linoleic, linolenic and arachidonic acids were the major PUFAs. Furthermore, carcasses

of transgenic pigs had near the optimum ratio of 1:1:1 for SFA:MUFA:PUFA as recommended by National Research Council (1988). Therefore, both in mass and profile, meat from transgenic pigs contains less lipid that is less hyperlipidemic and hypercholesterolemic to consumers and more consistent with contemporary human diet-health guidelines. Cholesterol content of the ground carcasses (Table 1) either was not different or increased slightly when comparing the controls to the transgenic pigs (68.71 C; 67.87 T-oGH; 69.85 T-hGH; 77.18 T-bGH, mg 100 g^{-1}).

Lean tissue composition

Loin eye area was similar in size between the T-bGH and control pigs (32.37 vs. 33.91 cm^2). This suggests that the major compositional alterations in T-pigs is a result of major reductions in fat and not the result of increases in muscle tissue accretion resulting from hypertrophy. Intramuscular fat (Table 1) was also reduced in the lean tissue (*longissimus* muscle) in T-bGH, T-oGH, and T-hGH pigs (52, 67, and 60%, respectively) compared to controls. This reduction in intramuscular fat was associated with reductions in SFA (61, 80, and 63%), MUFA (69, 88, and 78%) and PUFA (29, 41, and 21%) for T-bGH, T-oGH and T-hGH pigs (Fig. 2), respectively, compared to sibling controls. Cholesterol content of the lean tissue (Table 1) either was not different or increased slightly when comparing the controls to the transgenic pigs (48 vs 52 mg 100 g^{-1}). Although the role of dietary cholesterol intake in humans is questionable, the general recommendation is to limit intake to 100 mg 1000 $kcal^{-1}$, not to exceed 300 mgd^{-1} (American Heart Association, 1986). Lean tissue from transgenic pigs also had near the optimum ratio of 1:1:1 for SFA:MUFA:PUFA as recommended by National Research Council (1988).

Meat tenderness

In spite of the dramatic reductions in separable and intramuscular fat throughout the carcasses and primal cuts of the T-bGH pigs, evaluation of tenderness using the Warner-Bratzler shear test indicated that *longissimus* (loin) muscle tenderness (Table 1) was not different between the T-bGH and C pigs. Actual processing properties, yields, and products have not been determined to date for meat from genetically engineered pigs.

There is no question that either method, i.e. exogenous administration of somatotropin, or microinjecting foreign DNA into the pronucleus of fertilized ova in pigs, improves productive traits and carcass compositions. With major emphasis on lean tissue accretion and less lipid deposition, either technology can be used with great success. Responses for increased protein accretion would favor exogenous pST administration over transgene technology, whereas, responses for increased lipid depletion would favor transgene technology. Both offer approaches for progress in meeting consumer demands for lean meat.

CONCLUSIONS

Potential for manipulation of growth and composition of farm animals has never been greater than at present due to the wide array of strategies for altering the balance between lean and fat. Although major progress is being made using biotechnological techniques, such as genetic engineering, much more needs to be accomplished. Both pST administration and transgene technology offer tremendous means for endogenously reducing carcass fat; however, eating quality and safety must not be sacrificed as leaner animals are developed. We are still a long way from fully understanding the integrated mechanisms resulting from the manipulation of growth and carcass composition as a result of these strategies. The significant decrease in waste fat will have major economic value to the meat/pork industry as well as to the consumer.

REFERENCES

American Heart Association. (1986). *Dietary guidelines for healthy american adult.* American Heart Association Circulation. 74, 1465A.

Bonamone, A. & Grundy, S. M. (1988). Effect of dietary stearic acid on plasma cholesterol and lipoprotein levels. *N. Engl. J. Med.*, **318**, 1244–1248.

Brinster, R. L., Chen, H. Y., Trumbauer, M. E., Yagle, M. K. & Palmiter, R. D. (1985). Factors affecting the efficiency of introducing foreign DNA into mice by microinjecting eggs. *Proc. Natl. Acad. Sci. USA*, **82**, 4438–4444.

Campbell, R. G., Johnson, R. J., King, R. H., Taverner, M. R. & Meisinger, D. J. (1990). Interaction of dietary protein content and exogenous porcine growth hormone administration on protein and lipid accretion rates in growing pigs. *J. Anim. Sci.*, **68**, 3217–3225.

Caperna, T. J., Steele, N. C., Komarek, D. R., McMurtry, J. P., Rosebrough, R. W., Solomon, M. B. & Mitchell, A. D. (1990). Influence of dietary protein and recombinant porcine somatotropin administration in young pigs' growth, body composition and hormone status. *J. Anim. Sci.*, **68**, 4243–4252.

Clark, S. L., Wander, R. C. & Hu, C. Y. (1992). The effect of porcine somatotropin supplementation in pigs on the lipid profile of subcutaneous and intermuscular adipose tissue and longissimus muscle. *J. Anim. Sci.*, **70**, 3435–3441.

Etherton, T. D., Donkin, S. S., & Bauman, D. E. (1995). Mechanisms by which porcine somatotropin (pST) decreases adipose tissue growth in growing pigs. In: *The biology of fat in meat animals*, eds. S. B. Smith & D. R. Smith. American Society of Animal Science, Chicago, IL, USA. pp. 53–69.

Keys, A., Anderson, T. & Grande, F. (1965). Serum cholesterol response to changes in the diet. IV. Particular saturated fatty acids in the diet. *Metabolism*, **14**, 776–787.

Machlin, L. J. (1972). Effect of porcine growth hormone on growth and carcass composition of the pig. *J. Anim. Sci.*, **35**, 794–800.

Mattson, F. H. & Grundy, S. M. (1985). Comparison of dietary saturated, monounsaturated and polyunsaturated fatty acids on plasma lipids and lipoproteins in man. *J. Lipid Res.*, **26**, 194–202.

McKeith, F. M. (1993). *The effect of repartitioning agents on carcass and meat quality*. Proc. 39th Intl. Congress of Meat Sci. & Techn. Calgary, Canada. pp 3–20.

National Research Council. (1988). *Designing foods. animal product options in the market place*. National Academy Press, Washington, D.C.

Palmiter, R. D. & Brinster, R. L. (1986). Germ-line transformation of mice. *Annu. Rev. Genet.*, **20**, 465–478.

Palmiter, R. D., Brinster, R. L., Hammer, R. E., Trumbauer, M. E., Rosenfeld, M. G., Birnberg, N. C. & Evans, R. M. (1982). Dramatic growth of mice that develop from eggs microinjected with metallothionein–growth hormone fusion genes. *Nature*, **300**, 611–615.

Pursel, V. G. & Rexroad, C. E. Jr. (1993). Status of research with transgenic farm animals. *J. Anim. Sci.*, **71**(Suppl.3), 10–19.

Pursel, V. G., Solomon, M. B., & Wall, R. J. (1996). Genetic engineering of swine. In: *Advances in swine in biomedical research*, eds. M. E. Tumbleson & L. B. Schook. Plenum Publishing Corp., pp. 189–206.

Solomon, M. B., Campbell, R. G. & Steele, N. C. (1990). Effect of sex and exogenous porcine somatotropin on *longissimus* muscle fiber characteristics of growing pigs. *J. Anim. Sci.*, **68**, 1176–1182.

Solomon, M. B., Campbell, R. G., Steele, N. C. & Caperna, T. J. (1991). Effects of exogenous porcine somatotropin administration between 30 and 60 kg on *longissimus* muscle fiber morphology and meat tenderness of pigs grown to 90 kg. *J. Anim. Sci.*, **69**, 641–645.

Solomon, M. B., Caperna, T. J., Mroz, R. J. & Steele, N. C. (1994*a*) Influence of dietary protein and recombinant porcine somatotropin administration in young pigs: III. Muscle fiber morphology and meat tenderness *J. Anim. Sci.*, **72**, 615–621.

Solomon, M. B., Pursel, V. G., Paroczay, E. W. & Bolt, D. J. (1994*b*) Lipid composition of carcass tissue from transgenic pigs expressing a bovine growth hormone gene *J. Anim. Sci.*, **72**, 1242–1246.

Turman, E. J. & Andrews, F. M. (1955). Some effects of purified anterior pituitary growth hormone on swine. *J. Anim. Sci.*, **14**, 7–15.

Wall, R. J., Hawk, H. W. & Nel, N. (1992). Making transgenic livestock: Genetic engineering on a large scale. *J. Cell. Biochem.*, **49**, 113–120.