Annu. Rev. Nutr. 1990. 10:213–32



# NUTRIENT PARTITIONING BY TRANSGENIC ANIMALS[1]

*Norman C. Steele and Vernon G. Pursel*

Livestock and Poultry Sciences Institute, Agricultural Research Service, United States Department of Agriculture, Beltsville, Maryland 20705

KEY WORDS: growth, transgene manipulations, diet, swine



## CONTENTS



## PREFACE

The intent of this chapter is to describe, to the extent possible, the expected change in nutrient requirements resulting from transgene manipulations. As such, dietary components associated with growth processes are of primary importance, and only the elements directly involved with growth and develop-

[1]The US government has the right to retain a nonexclusive, royalty-free license in and to any copyright covering this paper.

ment are pertinent, specifically the structural growth hormone (GH) transgene manipulations. This technology must be considered in its infancy compared with the knowledge base in exogenous hormone treatments, and much of what can be assumed regarding nutritional requirements is based on deduction from exogenous GH treatment.

At a basic and superficial level, growth of plants and animals can be considered the result of hyperplasia of differentiated cells and the subsequent hypertrophy of these cells. Once a tissue is differentiated and relatively static with respect to DNA content, growth is the result of competitive hypertrophy of various tissue types, based on the availability of nutrients in the extracellular space. The latter forms the basis for the nutrient partitioning concept. Postdifferentiation growth of mammals occurs by a process that relates to the essentiality of a given tissue toward survival of the species. Sir John Hammond (41) described the concept underlying nutrient partitioning, and these concepts are paraphrased in the model shown in Figure 1. According to the model, neural and bone tissue growth occurs with a relatively high priority established by genetic composition of the species, and growth of these tissues is sustained even in marginal nutritional environments. Muscle and adipose tissue, once differentiated, hypertrophy in response to environmental factors, including nutritional environment, only to the degree permitted by genetic composition. Livestock production practices have recently focused on the maximum yield of lean tissue with modest accretion of body fat. Within the specialty of animal nutrition, one often assumes that feeding standards have been established to fully exploit the genetic capacity for lean tissue growth. This assumption formed the basis for our interest in exogenous GH treatment and growth hormone transgene manipulations.



## THEORY OF NUTRIENT PARTITIONING

According to the Hammond model (41), the relative accretion of muscle and adipose tissues could be altered either by decreasing the slope of the line (i.e. change of tissue growth priority at a given plane of nutrition-partitioning), or by changing the hierarchy between muscle and adipose tissue growth priority at a given concentration of nutrients in the extracellular environment (i.e. repartitioning). Repartitioning strategies rely on pharmacologic site-specific alterations of metabolism. Examples include beta-adrenergic agonists (possibly), thyroid-active compounds, and steroid implants. Partitioning strategies can be grouped into techniques referred to as genetic tools. The genetic tools most recognized for the modification of animal growth are the gene-optimization procedures used by animal breeders. A classic example is the Beltsville selection experiment for or against subcutaneous fat thickness in swine (23); this experiment simultaneously affected muscle tissue deposition

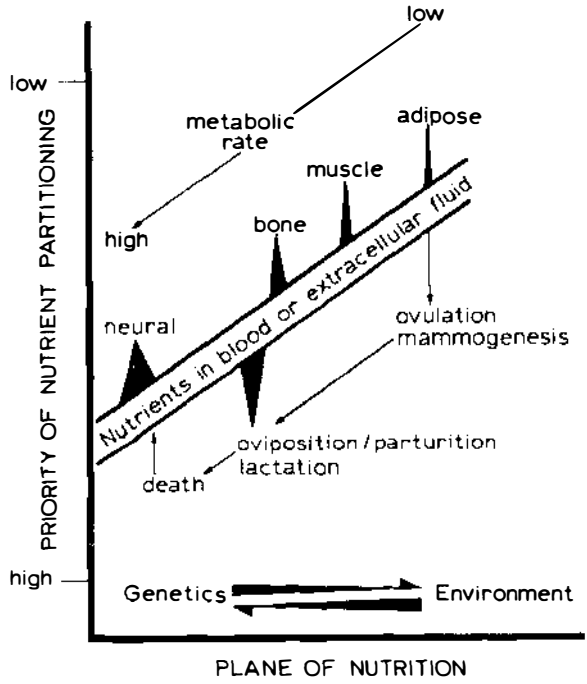


*Figure 1* Hammond (41) model of nutrient partitioning. Boldness of the arrow relates to the hierarchial need of tissue sustenance for survival of the species.

and skeletal growth. Recently, a strain of swine has been characterized with an apparently infinite capacity for protein growth, as compared with typical commercial strains (15). In this genotype, appetite was concluded to limit protein accretion. These examples emphasize that gene-optimization techniques can markedly influence both adipose and muscle tissue priorities for nutrient use. Such techniques as practiced over many centuries of livestock domestication are characteristically very slow and costly but highly effective and generally regarded as safe with respect to environmental concerns. Unfortunately, the lack of knowledge regarding specific genes involved in growth and development processes and the inability to manipulate such genes within large populations of animals prevent rapid progress in reducing fat and increasing lean tissue deposition using gene-optimization procedures.

Recent progress in foreign gene insertion and expression (39, 40) has led to the production of agriculturally important transgenic animals. The technology has the potential not only to alter the slope of the nutrient partitioning priority line but also to alter the absolute genetic ceiling for both muscle protein and fat accretion by the only method capable of truly altering genetic composition. However, elucidation of the mechanisms involved in the control of gene

expression and the subsequent impact on growth and development as well as overcoming the remarkable negative public image of this technique will require considerable research investment and a lengthy education of the consuming public.

Yet another technique available to alter nutrient partitioning priority would be to increase preferentially the efficiency of muscle protein deposition, assuming that full genetic potential has been underestimated and constrained within normal physiologic processes. In effect this strategy involves "gene amplification" and describes efforts that manipulate rate and composition of growth by exogenous GH administration. With strategic application of species-specific GH to induce supraphysiologic concentrations of "primary growth stimulants" (29), those genes normally involved with growth processes are activated more completely and over longer periods of time, thereby permitting a species to express more fully its genetic potential for protein deposition. According to the Hammond (41) model, pigs the size of steers, or steers the size of elephants, could not result from GH-induced gene amplification for protein deposition independent of proportionate increases in skeletal growth. However, pigs with feed efficiency comparable to that of broiler chickens and dairy animals with 25% greater-than-average milk yields could enter the animal production system. GH-induced growth alteration should not be construed as a violation of animal rights but rather as allowing the expression of genetic potential.

An obvious example of naturally occurring gene amplification is the sex effect difference in rate and composition of gain comparing the intact male with the female pig (15, 17). Genetic composition does not differ greatly in this example, but expression of genetic potential for protein deposition is apparently constrained in the female by the lack of androgen stimulation. The quantitative definition of genetic potential for lean tissue growth is of paramount importance to animal nutritionists, because both rate and composition of lean growth defines the nutrient requirement at a tissue level. Once defined, feeding recommendations can be formulated that address this requirement and are corrected for obligatory digestion losses. Whether such formulations are currently practical is not important; however, once the potential is known, the economics of practical diet formulations can be evaluated objectively.

While the mechanism(s) of action of GH are not fully understood, this hormone is known to have direct effects on metabolism in some cells, while indirect actions on other tissues and cells are mediated through secondary growth factors or somatomedins (i.e. insulinlike growth factors, IGF-I). Many anabolic effects of GH in animals are mediated through the IGFs. Several excellent reviews are available concerning IGF receptors (22, 68), biological activity, and molecular biology (29, 77). Although a great deal of

information is available concerning biochemical aspects of these polypeptide hormones, there is a dearth of information regarding their functions in rapidly growing, meat-producing animals. The direct influence of GH on lipid metabolism in adipose tissue has been studied extensively in vitro in rats by Goodman and coworkers (31, 32) and in pigs by Walton et al (83–85). These studies have shown that GH decreases glucose utilization, inhibits lipid synthesis, and enhances lipid turnover, and these actions partly explain the significant reduction in fat depots in GH-treated animals. These effects are observed in the presence of GH in vitro under certain circumstances and appear to require glucocorticoids. Goodman (31) suggested, however, that normal circulating levels of GH are sufficient to attain maximal GH effects in adipose tissue. Therefore, the observed reductions in body fat cannot be explained totally by treatment with supraphysiologic levels of GH; thus other regulatory mechanisms need investigation. Bauman et al (2) revitalized interest in the homeorhetic mechanism of hormone action, which is pertinent to GH. According to the concept, a hormone, such as GH, elicits changes at multiple sites and coordinates the adaptation of the whole organism to a new physiologic state. Therefore, inhibitory effects on adipose tissue growth occur in synchrony with the stimulation of muscle protein accretion. Nutrients destined as storage lipid are coordinated in use to support protein synthesis.



## TRANSGENIC PROCEDURES AND TRANSGENE EXPRESSION

### *Methods of Transferring Genes*

The predominant method used to transfer cloned genes into animals is direct microinjection into the pronuclei of fertilized eggs. Although microinjection of mouse pronuclei is readily performed, direct application to other species was impeded by the opacity of the egg cytoplasm. Pronuclei of rabbit and sheep eggs can be seen readily using differential interference contrast (DIC) microscopy. However, pig and cow eggs must be centrifuged at 15,000 g for 5 min to induce stratification of the cytoplasm before the pronuclei are visible with use of DIC microscopy (82).

The mechanism by which injected DNA integrates into a chromosome is unknown. Usually the DNA integrates in a single site, but multiple integrations can occur. Approximately 70% of transgenic mice produced by microinjection carry the transgene in all cells and the remaining 30% are mosaic, presumably owing to integration of the gene at some subsequent cleavage stage after microinjection (89). Frequently, injected DNA results in multiple copies of the gene integrating either in head-to-tail or head-to-head array (34, 38, 75).

The efficiency of transgene integration is much lower for farm animals than

for mice. In mice, approximately 10 to 15% of the microinjected eggs develop to term, and approximately 25% of weaned mice are transgenic (11). In contrast, only approximately 8% of the microinjected sheep and swine eggs result in young at birth, and only about 10% of those are transgenic (63, 70). Some of the important parameters that influence the frequency of integration have been described for mice (11, 33, 43).

Infection of embryos with retrovirus has been successful in several species (5, 44, 46, 59, 74, 78). This technique is receiving considerable attention because it offers several advantages over microinjection in certain applications. Principal advantages are: (*a*) single copies of the gene integrate without rearrangement at the site of integration; and (*b*) retroviral DNA integrates into a high percentage of embryos that can be infected by exposure to high concentrations of viral stock, by coculture with infected cells in vitro, or in chickens, by microinjection into the blastodisk. The disadvantages are: (*a*) producing a retrovirus carrying the transgene creates added work; (*b*) a size limit on the gene to be inserted into the retrovirus; (*c*) a high incidence of mosaicism in resulting transgenics necessitates extensive outbreeding to establish pure transgenic lines; and (*d*) unresolved problems remain with expression of the transgene (45).

The third method of introducing genes into the germ line involves transfer into embryonic stem cells in culture and then incorporating these transgenic stem cells into an embryo. The advantage of this procedure is that a particular genotype can be selected in vitro before introduction of the stem cells into the embryo. This technique is thus far the only one that provides the ability for site-specific insertion of a transgene by homologous recombination (18). Thus far, only transgenic mice have been produced by this method.

## *Transfer of Growth-Related Genes*

While transgenesis will undoubtedly be used to investigate a wide range of important areas of nutrition in the future, this review is limited to the growth-related genes that have been transferred into mice and several other species as shown in Table 1. One might expect that transfer of structural genes encoding growth-related peptide hormones along with 5' regulatory flanking sequences might produce the most desirable gene expression characteristics. However, neither *rGH* and *hGH* structural genes with their own 5' regulatory sequences nor the *rGH* promoter ligated to the *hGH* structural gene resulted in enhancing the growth of transgenic mice harboring these transgenes (38, 47, 81). Palmiter and coworkers (57, 58) recognized that the normal GH regulatory mechanisms in the hypothalamic-pituitary axis probably prevented secretion of excess GH from the pituitary. To circumvent this normal regulatory scheme, they pioneered the use of a fusion gene composed of regulatory

**Table 1** Growth-related fusion genes transferred into animals

| Fusion gene | Integration | Expression | Germline transmission | Reference |
|---|---|---|---|---|
| *(CATTLE)* | | | | |
| MMTV-bGH | yes | no | no | 73 |
| *(MICE)* | | | | |
| AL-hGH | yes | yes | no | 60 |
| H2K-hGH | yes | yes | yes | 52 |
| MT-bGH | yes | yes | yes | 36 |
| MT-hGH | yes | yes | yes | 58 |
| MT-rGH | yes | yes | yes | 57 |
| MT-oGH | yes | yes | yes | 56 |
| MT-pGH | yes | yes | yes | 79 |
| MT-hGRF | yes | yes | yes | 37 |
| MT-hIGF-I | yes | yes | yes | 48 |
| PEPCK-bGH | yes | yes | yes | 50 |
| rGH-hGH | yes | yes | yes | 47 |
| *(SHEEP)* | | | | |
| MT-hGH | yes | no | no | 39 |
| MT-bGH | yes | yes | no | 67, 71 |
| MT-oGH5 | yes | no | no | 53 |
| MT-oGH9 | yes | yes | no | 53 |
| MT-hGRF | yes | yes | yes | 70, 71 |
| TF-bGH | yes | yes | no | 70, 71 |
| AL-hGRF | yes | yes | no | 70 |
| *(SWINE)* | | | | |
| ALB-hGRF | yes | yes | no | 65 |
| CMV-pGH | yes | yes | no | K. M. Ebert (unpublished) |
| MT-bGH | yes | yes | yes | 66, 67 |
| MT-hGH | yes | yes | yes | 7, 39 |
| MT-hGRF | yes | yes | yes | 9, 61, 66 |
| MT-hIGF-I | yes | yes | no | 66 |
| MT-pGH | yes | yes | yes | 80 |
| MLV-pGH | yes | yes | no | K. M. Ebert (unpublished) |
| MLV-rGH | yes | yes | no | 26 |
| PEPCK-bGH | yes | yes | yes | 87, 88 |
| PRL-bGH | yes | yes | yes | 62 |
| *(FISH)* | | | | |
| MT-hGH | yes | yes | no | 8, 21, 90 |
| hGH | yes | no | no | 24 |

sequences from mouse metallothionein *(mMT)* gene ligated to the structural *rGH* gene to direct rGH production to an ectopic site, which resulted in the dramatically fast-growing "super" mouse (57, 58). Subsequent investigations have been extended to other regulator-promoter sequences, other growth-related fusion genes, and several other species (Table 1).

## *Expression of Integrated Genes*

INCIDENCE OF EXPRESSION Approximately 70% of the transgenic mice and pigs that have been tested expressed the integrated gene when the structural gene was composed of genomic DNA (36, 37, 57, 58, 66). Failure to express the transgene may be the result of integration in an inactive chromosomal locus or alteration of gene sequences during the integration process. In some cases, the presence of introns in the structural region may greatly influence whether the gene is expressed. In transgenic mice, Brinster et al (10) found that genes without introns were expressed with a lower frequency and at a lower level than were the same genes with introns.

Possibly, the lack of introns in the *MT-pGH, MLV-pGH,* and *CMV-pGH* constructs was responsible for the low percentage of pigs expressing the gene product [17% according to (80); or 10% K. M. Ebert, unpublished observations]. Only two of eight pigs and one of seven lambs expressed the *MT-hGRF* gene, which contained only the first intron (66, 71). The incidence of expression of the same fusion genes was much higher in transgenic mice than in pigs; 13 of 18 mice expressed *MT-pGH* (79), and 11 of 14 mice expressed *MT-hGRF* (37). Reasons for this discrepancy in incidence of expression among species are unknown.

The *MT* promoter directed expression of fusion genes to the expected tissues in transgenic pigs. However, the concentrations of *bGH* mRNA in pig tissues were considerably lower than in tissues of transgenic mice harboring the same fusion genes (36, 58). In pigs and sheep *MT-bGH* genes produced high levels of messenger RNA in liver, kidney, testis, adrenal, and pancreas, with low levels in several other tissues (66, 71). Ebert et al (26) reported *rGH* mRNA concentration was high in spleen, lung, colon, and jejunum, with lower concentrations in the kidney, lymph nodes, and bone marrow of a transgenic pig with *MLV-pGH*. The *PEPCK-bGH* gene was expressed only in the liver and kidney in mice (50), whereas in *PEPCK-bGH* transgenic pigs the *bGH* mRNA was detected only in the liver in all but one pig that also expressed low levels in kidney, small intestine, and pituitary (88).

LEVEL OF GENE EXPRESSION Since fusion genes integrate in a different locus in each transgenic founder, the rate of gene transcription is probably determined by the general activity at the locus of integration and the characteristics of enhancer sequences in genes located on either side of the fusion

gene. As a consequence, the level of gene expression varies greatly among transgenic animals that integrate the same fusion gene. In *MT-hGH* transgenic mice, plasma concentrations varied among mice from 10 to 64,000 ng hGH/ml (58), whereas the same construct in pigs resulted in levels varying from 14 to 4551 ng hGH/ml (51). The concentrations of serum bGH in mice expressing the PEPCK-bGH transgene ranged from 5 to more than 2300 ng/ml (50).

Plasma concentrations of hGH were unrelated to the number of gene copies per cell in *MT-hGH* transgenic pigs (39). However, Miller et al (51) found that in transgenic pigs with *MT-bGH*, the concentration of bGH in plasma both at birth or at 30 to 180 days of age was positively correlated ($p<0.05$) with the number of gene copies per cell.

In transgenic mice harboring fusion genes with the *MT* promoter, concentrations of hGH and bGH in plasma were elevated 3- to 100-fold after zinc was added to their drinking water (58). In contrast, addition of 1000 to 3000 ppm zinc to the feed of transgenic pigs resulted in little more than a doubling of the bGH concentration in plasma (63). Polge et al (62) reported that several transgenic pigs with a *PRL-bGH* fusion gene released bGH episodically after being injected with thyrotropin-releasing hormone (TSH) or receiving infusions of a dopamine antagonist, sulpiride. Both studies with pigs demonstrated that the promoter sequences of fusion genes can respond to induction.

While these examples demonstrate that several promoters respond to induction, the ability to modulate expression by either raising or lowering the level of expression is highly desirable. McGrane et al (50) reported that bGH concentrations in serum could be reduced by 95% in transgenic mice with *PEPCK-bGH* by feeding the mice a high-carbohydrate diet for one week. Conversely, feeding a high-protein diet stimulated enhanced expression of the transgene. Although these results are extremely promising, regulation of expression in *PEPCK-bGH* transgenic pigs was insufficient to prevent them from developing many of the same health problems (see below) encountered in *MT-bGH* pigs (88).

PERFORMANCE AND PHYSIOLOGIC CHARACTERISTICS The enhanced growth rate and body size of transgenic mice that expressed foreign GH genes (57, 58) provided both the impetus for transfer of similar fusion genes into other species and the expectation that the rate of growth might be greatly enhanced. This expectation was not realized in the founder population of *MT-hGH* and *MT-bGH* transgenic pigs and *MT-bGH* transgenic lambs, even though convincing evidence indicates the transgenic animals produced a biologically active form of GH. Transgenic pigs expressing the hGH gene product rarely had detectable concentrations of plasma pGH (51); this fact indicates the negative feedback mechanism was functioning. Furthermore,

IGF-I concentrations were 2- to 7-fold higher in pigs transgenic with *hGH*, *bGH*, or *rGH* than in littermate control pigs (26, 51), which also indicates that foreign GH exerted a biologic effect by binding to GH receptors of hepatocytes to stimulate IGF-I synthesis.

The *MT-hGH* and *MT-bGH* founder transgenic pigs were fed a diet containing only 16% protein, a level that may not have provided sufficient protein for them to gain faster than their littermate controls. Recent studies using pigs injected with exogenous pGH indicate that maximal growth rate is attained only if the diet contains adequate protein, particularly, lysine (30, 54). In subsequent studies, the levels of dietary protein and lysine were increased during the 30- to 90-kg growth period, causing the *MT-bGH* transgenic pigs to gain weight 16.5% faster than sibling control pigs (64). In addition, Vize et al (80) reported that a transgenic pig expressing *MT-pGH* gained 492 g per day faster than did littermate control pigs during the 20- to 90-kg growth period.

Several recent studies of pigs treated with exogenous pGH have revealed that appetite depression accompanies elevated GH levels in pigs (13, 28). This finding may explain why growth rates of GH-treated pigs are not increased as dramatically as in transgenic mice or in rats with a GH-secreting tumor, which have enhanced feed intake (49). Compared with littermate or sibling controls, feed intake was depressed 20% in *MT-bGH* founder and 17% in *MT-bGH* second generation transgenic pigs fed ad libitum (66). These results are comparable, respectively, to a 14% and 17% depression in feed intake reported for pigs injected with pGH (13, 28).

*MT-bGH* founder transgenic pigs were 16% more efficient and *MT-bGH* second generation transgenic pigs were 18% more efficient in converting feed into body weight gain than were littermate or sibling controls (66). Similar improved feed efficiencies of 23% (13) and 25% (28) were reported for pigs injected with exogenous pGH compared with littermate controls.

Elevated concentrations of GH in pigs expressing *MT-hGH* and *MT-bGH* transgenes produced marked repartitioning of nutrients away from subcutaneous fat and into other carcass components, including muscle, skin, bone, and certain organs. Ultrasonic estimates or slaughter measurements of backfat thickness at the tenth rib of *hGH* and *bGH* transgenic pigs at approximately 90 kg body weight averaged 7.0 and 7.9 mm, respectively, whereas littermate control pigs averaged 18.5 and 20.5 mm, respectively (40, 66). Similar reductions in backfat were found for *PEPCK-bGH* transgenic pigs (88). Additionally, the backfat measurements do not adequately reflect the lack of subcutaneous fat in the *MT-bGH* transgenic pigs because total carcass lipid averaged only 3.7% compared with 19.8% for sibling controls (V. G. Pursel and M. B. Solomon, unpublished data).

Ebert et al (26) reported that by 9 months of age, a transgenic boar with *MLV-rGH* was 26% heavier, and its linear bone growth of fore and hind limbs

was greater than that of a littermate control boar. In contrast, at 8 and 10 months of age, *MT-hGH* and *MT-bGH* transgenic pigs had not grown to a larger body size, nor were femur, tibia, or humerus longer in four *MT-bGH* transgenic pigs than in sibling controls (66). Additional investigation is required to determine whether this difference is due to structural differences of bGH and rGH that affect binding to GH receptors in epiphyseal chondrocytes or to some other physiologic factor, or whether the single *MLV-rGH* transgenic boar represents a unique occurrence.

Transgenic pigs expressing *MT-hGH* and *MT-bGH* were moderately hyperglycemic, averaging 10 to 40 mg/dL above littermates, and insulin concentrations in fasted *MT-bGH* transgenics were elevated about 20-fold above those of siblings (66). Pigs injected daily with exogenous pGH had average increases in serum glucose ranging from 8 to 48% and concentrations of serum insulin that were two to sevenfold higher than that of control pigs (13, 14, 28). In comparison, a *MLV-rGH* transgenic pig had glucosuria and consistently had serum glucose levels more than threefold higher than normal (26).

Pigs expressing *GH* transgenes exhibited a number of notable health problems, including arthropathy, susceptibility to stress, peptic ulcers, lethargy, anestrus in gilts, and lack of libido in boars (66, 67, 88). Arthropathy characteristic of osteochondritis dissecans was also observed in the *MLV-rGH* transgenic pig (26) and in some pigs treated with exogenous pGH for 57 days (28). In contrast, no increase in the incidence of these pathologic conditions was observed in nonexpressing *MT-hGH* or *MT-bGH* transgenic pigs (67) or in *PRL-bGH* transgenic pigs that expressed only low levels of bGH (62).

Many of the health problems observed in pigs exposed to high concentrations of GH are quite prevalent in the general swine population but at a lower incidence and with less severity. Several necropsy surveys indicate that 10 to 30% of slaughter-weight animals have gastric ulcers (55) and up to 90% of rapidly growing pigs have lesions of osteochondrosis (20, 69), which leads to degenerative joint disease, the major cause of lameness in swine. Additional investigation is required to determine whether these ailments would be exhibited in pigs expressing GH fusion genes if the foundation stock was not predisposed to such conditions.

Reproductive capacity of transgenic pigs expressing *MT-hGH* and *MT-bGH* genes was seriously impaired. Gilts failed to exhibit estrus, and at necropsy their ovaries were devoid of corpora lutea or corpora albicans (V. G. Pursel, unpublished data). Boars totally lacked libido; spermatozoa were recovered by electroejaculation or by flushing them from the epididymis at necropsy to use for artificial insemination to obtain germ line transmission of the transgene (67). Plasma concentrations of estrone sulphate (E1S) did not increase between 80 and 125 days of age in boars expressing a *MT-bGH* transgene,

whereas E1S levels tripled in sibling control boars at this stage of sexual development (35). Profiles of follicle-stimulating hormone and luteinizing hormone in plasma were similar for transgenic and sibling control gilts or boars, a finding that suggests the boar's' decreased libido may be more closely related to altered steroid metabolism than to pituitary function.

A major difference between a transgenic pig with elevated GH levels and a normal pig injected daily with exogenous GH is that, in the normal pig, GH level is elevated episodically, whereas in the transgenic pig, GH level is elevated continuously (51). Rats infused continuously with a high concentration of GH do not attain a maximal rate of growth (72). Continuous exposure to elevated levels of GH may contribute to the multiple health problems observed in the *GH* transgenic pigs and also may prevent them from growing to their full potential. Use of promoters that permit expression of *GH* fusion genes only during the rapid growth phase or promoters that can induce the release of large episodic doses of GH may be essential to achieve only the positive aspects of elevated GH for pigs. Future research on growth regulation with transgenic pigs will most certainly be directed toward this goal, along with investigation on the potentials of IGF-I, GH receptors, and other structural genes involved in growth.



## NUTRIENT RECOMMENDATIONS FOR GROWTH-REGULATED TRANSGENIC ANIMALS

A stated requirement for a given nutrient represents a single point along a dose-response curve that when applied to a well-characterized animal population, can with some reliability achieve predictable growth performance or targeted composition of body weight gain. Transgenic animal populations represent a unique challenge to nutritionists because such animals are not well characterized with respect to the population in general because of the relatively small numbers of expressing transgenic animals available. More important, heterogeneity of each individual in a single transgene population (i.e. copy number, orientation, expression level) confounds characterization. Therefore, few citations can be referenced regarding nutrient requirements; however, several contemporary methods to deduce nutrient formulations can be offered. The following rationale is pertinent to *GH*-transgene animals where composition analysis has demonstrated a change of nutrient partitioning (39, 40, 63, 66, 67). No evidence is available to suggest that nutrient requirements will be altered in transgenic animals with nongrowth-related structural genes.

### *Amino Acids*

With genetic composition favoring lean tissue deposition, the objective of diet formulation should be the removal of nutrient constraints on growth and

development processes to maximize fully the genetic capacity of GH transgenic animals. Based on the current feeding recommendations for a genetically improved intact male, one might approximate a basal composition for a growth hormone transgenic pig. Using the concept of ideal protein (1; Table 2) as described by Wang & Fuller (86), investigators can calculate estimates of specific available amino acids to be provided via the diet to support lean tissue accretion. Table 3 lists the predicted amino acid requirements at several body weights based on the assumption that a superior genotype male pig and *GH* transgenic pig may be similar with respect to such requirements. These requirements were based on the use of a computer simulation model (3) utilizing a factorial approach to the prediction of swine growth. Basically, the process considers sex of the pig, body weight, feed ingredients, level of feed intake, environmental conditions, pen density, and similar practical considerations. Specific ingredient sources to satisfy these demands rely on a databank of feed ingredients of known composition and amino acid availability. Application of information to a specific diet formulation will not be attempted. As protein deposition is a function of energy intake with a diet otherwise balanced in amino acid profile (4, 12, 16), a complex relationship emerges between intake, dietary energy density, and amino acid profile. A change in either feeding level or dietary protein level will influence protein deposition, assuming that no specific amino acid deficiencies exist, whereas limitation of one or several amino acids would limit protein deposition to protein intake. Unfortunately, the response of swine to dietary energy varies with age, sex, and genotype, returning one to the issue of characterization of animal populations.

**Table 2** Proposed balance of essential amino acids relative to lysine for pigs from birth to 90 kg (1)[a,b]

| Amino acid | Ratio percent |
|---|---|
| Lysine | 100 |
| Methionine | 25 |
| Methionine + Cystine | 50 |
| Threonine | 60 |
| Tryptophan | 14 |
| Isoleucine | 54 |
| Leucine | 100 |
| Histidine | 33 |
| Phenylalanine | 48 |
| Phenylalanine + Tyrosine | 96 |
| Valine | 70 |

[a] Expressed ratio is on available amino acid basis.
[b] Ratio of essential : nonessential is 40 : 60 to 60 : 40.

**Table 3** Predicted amino acid requirement (g/Megacal digestible energy) of a male pig from a genetically improved geneotype at various live weights (3)

| Amino acid | Live weight (kg) | | |
|---|---|---|---|
| | 20 | 50 | 90 |
| Lysine | 2.93 | 2.05 | 1.84 |
| Methionine | 0.88 | 0.56 | 0.54 |
| Methionine + Cystine | 1.30 | 0.88 | 0.80 |
| Threonine | 1.51 | 1.05 | 0.92 |
| Tryptophan | 0.46 | 0.29 | 0.29 |
| Isoleucine | 1.67 | 1.17 | 1.05 |
| Leucine | 2.64 | 1.17 | 1.05 |
| Histidine | 0.79 | 0.54 | 0.50 |
| Phenylalanine | 1.34 | 0.92 | 0.84 |
| Phenylalanine + Tyrosine | 2.51 | 1.76 | 1.59 |
| Valine | 1.84 | 1.26 | 1.13 |



Boyd et al (6) evaluated the factorial approach to the prediction of nutrient requirements, specifically lysine, of swine treated with exogenous pGH, which greatly accelerates protein accretion (13, 14, 25, 27, 28, 30). Table 4 lists the process used to arrive at a recommendation of lysine intake sufficient to support body protein deposition. Discrepancy from the recommendation for an improved genotype (Table 3) can be attributed to the higher rate of protein deposition observed with exogenous GH treatment. Integration of the ideal protein concept (Table 2) with the factorially derived lysine estimate allows calculation of all essential amino acids required to support the accelerated

**Table 4** Factorial calculation of the lysine requirement of pigs differing in body weight and anticipated protein accretion rates (6)

| Body weight, kg: | 20–55 | | 55–100 | |
|---|---|---|---|---|
| Protein accretion rate, g/day: | 110 | 160 | 130 | 235 |
| Maintenance: | | | | |
| Protein, g/day | 24 | 29 | 45 | 55 |
| Lysine, g/day | 1.54 | 1.86 | 2.88 | 3.52 |
| Net synthesis: | | | | |
| Lysine, g/day[a] | 7.04 | 10.24 | 8.23 | 15.04 |
| Absorbed lysine required, g/day[a] | 13.20 | 18.62 | 17.23 | 28.55 |
| Dietary lysine requirement, g/day[b] | 15.71 | 21.16 | 20.51 | 32.44 |
| Lysine: Mcal ME ratio | 2.04 | 3.15 | 1.83 | 3.57 |

[a] Assumed efficiency for maintenance and synthesis is 65%.
[b] Digestibility for each weight class was 84 and 88%, respectively.

growth. These values would exemplify the maximal amino acid requirements of a *GH* transgenic pig.

The above rationale is particularly appropriate for *GH* structural gene transgenic animals utilizing ubiquitous promotor sequences such as metallothionein and transferrin. The chimeric *PEPCK-bGH* line of swine (87, 88) is distinctly different. The logistics of formulating a protein-adequate, carbohydrate-free diet to maximize gene expression is not possible without the use of purified diets enriched with high levels of dietary fat and fiber. The sensitivity of the *PEPCK* promotor to specific amino acids may hold promise as a means of modulating gene expression.

## *Energy*

A consistent response observed in expressing *GH*-transgenic animals and those treated with exogenous pGH is the depression of voluntary appetite (13, 14, 27, 28). For an enriched amino acid diet formulation to provide benefit, energy must be sufficient to support accelerated protein deposition. Based on results of Campbell et al (12, 15–17) a diet with 3600 kcal digestible energy/kg fed ad libitum would be sufficient from the postweaning period to approximately 60 kg body weight. Thereafter, diet intake should be restricted approximately 15% (85% ad libitum) to keep energy intake proportionate to the decrease of protein deposition at heavier weights. Considering that swine diet formulations typically contain 3300 kcal digestible energy/kg, carbohydrate must be deleted for the purpose of adding dietary lipid. This modest adjustment would ensure that dietary energy density for pigs of any live weight is within the reasonable constraints of appetite and, combined with intake control in heavy animals, is appropriate for protein deposition potential.

## *Vitamins and Minerals*

The increase of ash deposition rate in exogenous pGH-treated swine (19) and the structural abnormalities noted in *hGH* and *bGH* transgenic swine (66) might indicate the need for adjustment of dietary calcium and other trace mineral levels. However, calcium, zinc, and copper concentration of bone (i.e. mg/g dry bone or per g ash) are not influenced (19). Therefore, deposition of specific bone structural elements appears proportionate to the increase of growth rate and corresponding increase of bone mass associated with muscle mass. A reasonable approach to satisfy this need would be to increase dietary calcium and available phosphorous at a constant ratio in direct relation to the expected increase of protein accretion (i.e. 30 to 50%). Other considerations relate to the sensitivity of the promotor sequence region of a specific gene sequence to trace elements.

Modulation of gene expression in the rodent by dietary zinc has been

reported by Palmiter et al (58) but was not evident in the *MT-hGH* swine transgenic model until extraordinary dietary zinc concentrations (2000 ppm) were supplied. Basal dietary zinc concentration differs considerably between rodent and swine diets, a possible explanation for the lack of controllable gene expression in swine. Likewise, dietary iron as a modulator of transferrin promotor activity of *Tf-bGH* swine might be anticipated, but sensitivity has not been examined. At present no rationale to increase or decrease dietary zinc and iron content can be stated. Recommended supplements containing 100 ppm of zinc and iron are appropriate.

Typical balanced diet formulations contain added vitamin D for rodents and swine. Vitamin D metabolite concentrations differ in serum of pigs treated with exogenous pGH (29a). For pigs consuming a diet fortified with 880 IU vitamin D/kg, sufficient substrate is available to satisfy requirements, and differences observed in vitamin D metabolites reflect either rate-limiting enzymes in the vitamin D-1,25-dihydroxy-vitamin D pathway or represent a concerted adaptation proportionate to long-bone growth. The lack of specific vitamin-deficiency syndromes in research animal populations would not indicate the need to further fortify diets in other vitamins.



## FUTURE RESEARCH OPPORTUNITIES

The rapid evolution of transgenic animal germ lines will make available resources for nutritionists to ask pertinent questions regarding nutrient limitations to metabolic processes. Obstacles in the field of molecular biology (i.e. regulatable promotor sequences, understanding of gene copy number insertion mechanisms, ability to site-direct transgene insertion, improved expression efficiency) will certainly be overcome with expanded research efforts. With growth-regulated transgenic animals available in suitable numbers, specific questions regarding metabolic regulation can be addressed. In general, these efforts must be preceded by exhaustive characterization of such germlines.

### *Literature Cited*

1. Agricultural Research Council. 1981. *The Nutrient Requirements of Farm Livestock. No. 3, Pigs.* Tech. Rev. Summ., Agric. Res. Council. London. 307 pp.
2. Bauman, D. E., Eisemann, J. H., Currie, W. B. 1982. Hormonal effects on partitioning of nutrients for tissue growth: Role of growth hormone and prolactin. *Fed. Proc.* 41:2538–44
3. Black, J. L. 1987. Application of information on energy and amino acid utilization in the pig. See Ref. 76, pp. 119–29
4. Black, J. L., Campbell, R. G., Williams, I. H., James, K. J., Davies, G. T. 1986. Simulation of energy and amino acid utilization in the pig. *Res. Dev. Agric.* 3:121–45
5. Bosselman, R. A., Hsu, R.-Y., Boggs, T., Hu, S., Bruszewski, J., et al. 1989. Germline transmission of exogenous genes in the chicken. *Science* 243:533–35

6. Boyd, R. D., Wray-Cahan, D., Krick, B. 1988. Implications of somatotropin on nutrient partitioning of growing swine. *Proc. Cornell Nutr. Conf.*, Syracuse, NY, pp. 81–92
7. Brem, G., Brenig, B., Goodman, H. M., Selden, R. C., Graf, F., et al. 1985. Production of transgenic mice, rabbits and pigs by microinjection into pronuclei. *Zuchthygien* 20:251–52
8. Brem, G., Brenig, B., Horstgen-Schwark, G., Winnacker, E. -L. 1988. Gene transfer in tilapia *(Oreochromis niloticus)*. *Aquaculture* 68:209–19
9. Brem, G., Brenig, B., Muller, M., Kraublich, H., Winnacker, E.-L. 1988. Production of transgenic pigs and possible application to pig breeding. *Occas. Publ. Br. Soc. Anim. Prod.* 12:15–31
10. Brinster, R. L., Allen, J. M., Behringer, R. R., Galinas, R. E., Palmiter, R. D. 1988. Introns increase transcriptional efficiency in transgenic mice. *Proc. Natl. Acad. Sci. USA* 85:836–40
11. 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* 82:4438–42
12. Campbell, R. G. 1987. Response of the growing pig to energy intake. See Ref. 76, pp. 5–17
13. Campbell, R. G., Steele, N. C., Caperna, T. J., McMurtry, J. P., Solomon, M. B., et al. 1988. Interrelationships between energy intake and endogenous porcine growth hormone administration on the performance, body composition, and protein and energy metabolism of growing pigs weighing 25 to 55 kilograms live weight. *J. Anim. Sci.* 66: 1643–55
14. Campbell, R. G., Steele, N. C., Caperna, T. J., McMurtry, J. P., Solomon, M. B., et al. 1989. Interrelationships between sex and exogenous growth hormone administration on performance, body composition and protein and fat accretion of growing pigs. *J. Anim. Sci.* 67:177–86
15. Campbell, R. G., Taverner, M. R. 1988. Genotype and sex effects on the relationship between energy intake and protein deposition in growing pigs. *J. Anim. Sci.* 66:676–86
16. Campbell, R. G., Taverner, M. R. 1988. Genotype and sex effects on the responsiveness of growing pigs to exogenous porcine growth hormone (pGH) administration. *J. Anim. Sci.* 66 (Suppl. 1):257 (Abstr.)
17. Campbell, R. G., Taverner, M. R., Curic, D. M. 1985. Effects of sex and energy intake between 48 and 90 kg live weight on protein deposition in growing pigs. *Anim. Prod.* 40:497–503

17a. Campion, D. R., Hausman, G. J., Martin, R. J., eds. *Animal Growth Regulation*. New York/London: Plenum. 405 pp.

18. Capecchi, M. R. 1989. Altering the genome by homologous recombination. *Science* 244:1288–92
19. Caperna, T. J., Campbell, R. J., Steele, N. C. 1989. Interrelationships of exogenous growth growth hormone administration and feed intake level affecting various tissue levels of iron, copper, zinc and bone calcium of growing pigs. *J. Anim. Sci.* 67:334–38
20. Carlson, C. S., Hilley, H. D., Meuten, D. J., Hagan, J. M., Moser, R. L. 1988. Effect of reduced growth rate on the prevalence and severity of osteochondrosis in gilts. *Am. J. Vet. Res.* 49:396–402
21. Chourrout, D., Guyomard, R., Houdibine, L.-M. 1986. High efficiency gene transfer in rainbow trout (*Salmo gairdneri* Rich.) by microinjection into egg cytoplasm. *Aquaculture* 51:143–50
22. Czech, M. P. 1986. Structures and functions of the receptors for insulin and the insulin-like growth factors. *J. Anim. Sci.* 63 (Suppl. 2):27–38
23. Davey, R. J., Morgan, D. P., Kincaid, C. M. 1969. Response of swine selected for high and low fatness to a difference in dietary energy intake. *J. Anim. Sci.* 28:197–203
24. Dunham, R. A., Eash, J., Askins, J., Townes, T. M. 1987. Transfer of the metallothionein-human growth hormone fusion gene into channel catfish. *Trans. Am. Fish Soc.* 116:87–91
25. Easter, R. A. 1987. Nutritional requirements and repartitioning agents. *Proc. Univ. Ill. Pork Industry Conf.*, Urbana, IL, pp. 193–99
26. Ebert, K. M., Low, M. J., Overstrom, E. W., Buonomo, F. C., Baile, C. A. 1988. A Moloney MLV-rat somatotropin fusion gene produces biologically active somatotropin in a transgenic pig. *Mol. Endocrinol.* 2:277–83
27. Etherton, T. D., Wiggins, J. P., Evock, C. M., Chung, C. S., Rebhun, J. F., et al. 1987. Stimulation of swine growth performance by porcine growth hormone: Determination of the dose-response relationship. *J. Anim. Sci.* 64:433–43
28. Evock, C. M., Etherton, T. D., Chung, C. S., Ivy, R. E. 1988. Pituitary porcine growth hormone (pGH) and a recom-

binant pGH analog stimulate pig growth performance in a similar manner. *J. Anim. Sci.* 66:1928–41
29. Froesch, E. R., Schmid, Chr., Schwander, J., Zapf, J. 1985. Actions of insulin-like growth factors. *Annu. Rev. Physiol.* 47:443–67
29a. Goff, J. P., Caperna, T. J., Campbell, R. G., Steele, N. C. 1988. Interaction of porcine growth hormone (pGH) administration and dietary energy intake on circulating vitamin D metabolite concentrations in growing pigs. *J. Anim. Sci.* 66 (Suppl. 1):291–92 (Abstr.)
30. Goodband, R. D., Nelssen, J. L., Hines, R. H., Kropf, D. H., Thaler, R. C. et al. 1988. The effect of porcine somatotropin (PST) and dietary lysine level on growth performance and carcass characteristics of finishing swine. *J. Anim. Sci.* 66 (Suppl. 1):95 (Abstr.)
31. Goodman, H. M. 1988. The role of growth hormone in fat metabolism. In *Designing Foods*. Comm. Tech. Options Improve Nutr. Attrib. Anim. Prod. Natl. Res. Counc., pp. 163–172. Washington, DC: Natl. Acad. Press
32. Goodman, H. M., Coiro, V., Frick, G. P., Gorin, E., Grichting, G., et al. 1987. Effects of growth hormone on the rat adipocyte: A model for studying direct actions of growth hormone. *Endocrinol. Jpn.* 34(Suppl. 1):59–72
33. Gordon, J. W., Ruddle, F. H. 1983. Gene transfer into mouse embryos: Production of transgenic mice by pronuclear injection. *Methods Enzymol.* 101:411–33
34. Gordon, J. W., Ruddle, F. H. 1985. DNA-mediated genetic transformation of mouse embryos and bone marrow—a review. *Gene* 33:121–36
35. Guthrie, H. D., Pursel, V. G., Miller, K. F., Bolt, D. J., Palmiter, R. D. 1989. Effect of bovine growth hormone gene (bGH) expression, age and sex of pigs on follicle-stimulating hormone (FSH), luteinizing hormone (LH) and estrone sulphate (E1S) secretion during sexual maturation. *J. Anim. Sci.* 67(Suppl. 1):345–46 (Abstr.)
36. Hammer, R. E., Brinster, R. L., Palmiter, R. D. 1985. Use of gene transfer to increase animal growth. *Cold Spring Harbor Symp. Quant. Biol.* 50:379–87
37. Hammer, R. E., Brinster, R. L., Rosenfeld, M. G., Evans, R. M., Mayo, K. E. 1985. Expression of human growth hormone releasing factor in transgenic mice results in increased somatic growth. *Nature* 315:413–16
38. Hammer, R. E., Palmiter, R. D., Brinster, R. L. 1984. Partial correction of murine hereditary disorder by germ-line incorporation of a new gene. *Nature* 311:65–67
39. Hammer, R. E., Pursel, V. G., Rexroad, C. E. Jr., Wall, R. J., Bolt, D. J., et al. 1985. Production of transgenic rabbits, sheep and pigs by microinjection. *Nature* 315:680–83
40. Hammer, R. E., Pursel, V. G., Rexroad, C. E. Jr., Wall, R. J., Bolt, D. J., et al. 1986. Genetic engineering of mammalian embryos. *J. Anim. Sci.* 63:269–78
41. Hammond, J. A. 1952. Physiological limits to intensive production in animals. *Br. Agric. Bull.* 4:222–46
42. Heap, R. B., Prosser, C. G., Lamming, G. E., eds. 1989. *Biotechnology of Growth Regulation*. London: Butterworths. 286 pp.
43. Hogan, B. L. M., Costantini, F., Lacy, E. 1986. *Manipulation of the Mouse Embryo: A Laboratory Manual*. Cold Spring, NY: Cold Spring Harbor Laboratory. 332 pp.
44. Jaenisch, R. 1976. Germ line integration and Mendelian transmission of the exogenous Moloney leukemia virus. *Proc. Natl. Acad. Sci. USA* 73:1260–64
45. Jaenisch, R. 1988. Transgenic animals. *Science* 240:1468–74
46. Jahner, D., Haase, K., Mulligan, R., Jaenisch, R. 1985. Insertion of the bacterial *gpt* gene into the germ line of mice by retroviral infection. *Proc. Natl. Acad. Sci. USA* 82:6927–31
47. Lira, S. A., Crenshaw, E. B. III, Glass, C. K., Swanson, L. W., Rosenfeld, M. G. 1988. Identification of rat growth hormone genomic sequences targeting pituitary expression in transgenic mice. *Dev. Biol.* 85:4755–59
48. Mathews, L. S., Hammer, R. E., Brinster, R. L., Palmiter, R. D. 1988. Expression of insulin-like growth factor I in transgenic mice with elevated levels of growth hormone is correlated with growth. *Endocrinology* 123:2827–33
49. McCusker, R. H., Campion, D. R. 1986. Effect of growth hormone-secreting tumors on body composition and feed intake in young female Wistar-Furth rats. *J. Anim. Sci.* 63:1126–33
50. McGrane, M. M., deVente, J., Yun, J., Bloom, J., Park, E., et al. 1988. Tissue-specific expression and dietary regulation of a chimeric phosphoenolpyruvate carboxykinase/bovine growth hormone gene. *J. Biol. Chem.* 163:11443–51
51. Miller, K. F., Bolt, D. J., Pursel, V. G., Hammer, R. E., Pinkert, C. A., et al. 1989. Expression of human or bovine growth hormone gene with a mouse

metallothionein I promoter in transgenic swine alters the secretion of porcine growth hormone and insulin-like growth factor-1. *J. Endocrinol.* 120:481–88

52. Morello, D., Morre, G., Salmon, A.-M., Yaniv, M., Babinet, C. 1986. Studies in the expression of an H-2K/human growth hormone fusion gene in giant transgenic mice. *EMBO J.* 5:1877–83
53. Murray, J. D., Nancarrow, C. D., Marshall, J. T., Hazelton, I. G., Ward, K. A. 1989. The production of transgenic Merino sheep by microinjection of ovine metallothionein-ovine growth hormone fusion genes. *Reprod. Fertil. Dev.* 1: 147–55
54. Newcomb, M. D., Grebner, G. L., Bechtel, P. J., McKeith, F. K., Novakofski, J., et al. 1988. Response of 60 to 100 kg pigs treated with porcine somatotropin to different levels of dietary crude protein. *J. Anim. Sci.* 66(Suppl. 1):281 (Abstr.)
55. O'Brien, J. J. 1986. Gastric ulcers. In *Diseases of Swine*, ed. A. D. Leman, B. Straw, R. D. Glock, W. L. Mengeling, R. H. C. Penny, E. Scholl, pp. 725–37. Ames, Ia: Iowa State Press. 1212 pp. 6th ed.
56. Orian, J. M., Lee, C. S., Weiss, L. M., Brandon, M. R. 1989. The expression of a metallothionein ovine growth hormone fusion gene in transgenic mice does not impair fertility but results in pathological lesions in the liver. *Endocrinology* 124: 455–63
57. Palmiter, R. D., Brinster, R. L., Hammer, R. E., Trumbauer, M. E., Rosenfeld, M. G., et al. 1982. Dramatic growth of mice that develop from eggs microinjected with metallothionein-growth hormone fusion genes. *Nature* 300:611–15
58. Palmiter, R. D., Norstedt, G., Gelinas, R. E., Hammer, R. E., Brinster, R. L. 1983. Metallothionein-human GH fusion genes stimulate growth of mice. *Science* 222:809–14
59. Petters, R. M., Schuman, R. M., Johnson, B. H., Mettus, R. V. 1987. Gene transfer in swine embryos by injection of cells infected with retrovirus vectors. *J. Exp. Zool.* 242:85–88
60. Pinkert, C. A., Ornitz, D. M., Brinster, R. L., Palmiter, R. D. 1987. An albumin enhancer located 10 kb upstream functions along with its promoter to direct efficient, liver-specific expression in transgenic mice. *Genes Devel.* 1:268–76
61. Pinkert, C. A., Pursel, V. G., Miller, K. F., Palmiter, R. D., Brinster, R. L. 1987. Production of transgenic pigs harboring growth hormone (MTbGH) or growth hormone releasing factor (MThGRF) genes. *J. Anim. Sci.* 65 (Suppl. 1):260 (Abstr.)
62. Polge, E. J. C., Barton, S. C., Surani, M. H. A., Miller, J. R., Wagner, T. et al. 1989. Induced expression of a bovine growth hormone construct in transgenic pigs. See Ref. 42, pp. 189–99
63. Pursel, V. G., Bolt, D. J., Miller, K. F., Pinkert, C. A., Hammer, R. E., et al. 1990. Expression and performance in transgenic swine. *J. Reprod. Fertil.* 40 (Suppl.):In press
64. Pursel, V. G., Campbell, R. G., Miller, K. F., Behringer, R. R., Palmiter, R. D., et al. 1988. Growth potential of transgenic pigs expressing a bovine growth hormone gene. *J. Anim. Sci.* 66(Suppl. 1):267 (Abstr.)
65. Pursel, V. G., Miller, K. F., Bolt, D. J., Pinkert, C. A., Hammer, R. E., et al. 1989. Insertion of growth hormone genes into pig embryos. See Ref. 42. pp. 181–88
66. Pursel, V. G., Pinkert, C. A., Miller, K. F., Bolt, D. J., Campbell, R. G., et al. 1989. Genetic engineering of livestock. *Science* 244:1281–88
67. Pursel, V. G., Rexroad, C. E. Jr., Bolt, D. J., Miller, K. F., Wall, R. J., et al. 1987. Progress on gene transfer in farm animals. *Vet. Immunol. Immunopathol.* 17:303–12
68. Rechler, M. M., Nissley, P. 1986. The nature and regulation of the receptors for insulin-like growth factors. *Annu. Rev. Physiol.* 47:425–41
69. Reiland, S., Ordell, N., Lundeheim, N., Olsson, S. 1978. Heredity of osteochondrosis, body constitution and leg weakness in the pig. *Acta Radiol. Suppl.* 358:123–37
70. Rexroad, C. E. Jr., Hammer, R. E., Behringer, R. R., Palmiter, R. D., Brinster, R. L. 1990. Insertion, expression and physiology of growth-regulating genes in ruminants. *J. Reprod. Fertil.* Suppl. In press
71. Rexroad, C. E. Jr., Hammer, R. E., Bolt, D. J., Mayo, K. M., Frohman, L. A., et al. 1989. Production of transgenic sheep with growth regulating genes. *Mol. Reprod. Dev.* 1:164–69
72. Robinson, I. C. A. F., Clark, R. G. 1989. Growth promotion by growth hormone and insulin-like growth factor-I in the rat. See Ref. 42, pp. 129–40
73. Roschlau, K., Rommel, P., Andreewa, L., Zackel, M., Roschlau, D. et al. 1989. Gene transfer experiments in cattle. *J. Reprod. Fertil.* Suppl. 38:153–60
74. Salter, D. W., Smith, E. J., Hughes, S.

H., Wright, S. E., Crittenden, L. B. 1987. Transgenic chickens: Insertion of retroviral genes into the chicken germ line. *Virology* 157:236–40
75. Simons, J. P., Wilmot, I., Clark, A. J., Archibald, A. L., Bishop, J. O., et al. 1988. Gene transfer into sheep. *Bio/Technology* 6:179–83
76. Standing Committee on Agriculture. 1987. *Feeding Standards for Australian Livestock Pigs*. East Melbourne, Australia: CSIRO 226 pp.
77. Steele, N. C., Elsasser, T. H. 1989. Regulation of somatomedin production, relcase and mechanism of action. Sce Ref. 17a, pp. 295–315
78. van der Putten, H., Botteri, F. M., Miller, A. D., Rosenfeld, M. G., Fan, H. et al. 1985. Efficient insertion of genes into the mouse germ line via retroviral vectors. *Proc. Natl. Acad. Sci. USA* 82:6148–52
79. Vize, P. D. 1987. *Expression of porcine growth hormone in bacteria and transgenic animals*. PhD Thesis. Univ. Adelaide, Adelaide, South Australia. 129 pp.
80. Vize, P. D., Michalska, A. E., Ashman, R., Lloyd, B., Stone, B. A., et al. 1988. Introduction of a porcine growth hormone fusion gene into transgenic pigs promotes growth. *J. Cell Sci.* 90:295–300
81. Wagner, E. F., Covarrubias, L., Stewart, T. A., Mintz, A. 1983. Prenatal lethalities in mice homozygous for human growth hormone gene sequences integrated in the germ line. *Cell* 35:647–55
82. Wall, R. J., Pursel, V. G., Hammer, R. E., Brinster, R. L. 1985. Development of porcine ova that were centrifuged to permit visualization of pronuclei and nuclei. *Biol. Reprod.* 32:645–51
83. Walton, P. E., Etherton, T. D. 1986. Stimulation of lipogenesis by insulin in swine adipose tissue: antagonism by porcine growth hormone. *J. Anim. Sci.* 62:1584–95
84. Walton, P. E., Etherton, T. D., Chung, C. S. 1987. Exogenous pituitary and recombinant growth hormones induce insulin and insulin-like growth factor 1 resistance in pig adipose tissue. *Domest. Anim. Endocrinol.* 4(3):183–90
85. Walton, P. E., Etherton, T. D., Evock, C. M. 1986. Antagonism of insulin action in cultured pig adipose tissue by pituitary and recombinant porcine growth hormone: Potentiation by hydrocortisone. *Endocrinol.* 118:2577–81
86. Wang, T. C., Fuller, M. F. 1989. The optimal dietary amino acid pattern for growing pigs. 1. Experiments by amino acid deletion. *Br. J. Nutr.* 61:77–89
87. Wieghart, M., Hoover, J., Choe, S. H., McCrane, M. M., Rottman, F. M., et al. 1988. Genetic engineering of livestock-transgenic pigs containing a chimeric bovine growth hormone *(PEPCK/bGH)* gene. *J. Anim. Sci.* 66(Suppl. 1):266 (Abstr.)
88. Wieghart, M., Hoover, J. L., McGrane, M. M., Hanson, R. W., Rottman, F. M., et al. 1990. Production of transgenic swine harboring a rat phosphoenolpyruvate carboxykinase-bovine growth hormone fusion gene. *J. Reprod. Fertil.* Suppl. In press
89. Wilkie, T. M., Brinster, R. L., Palmiter, R. D. 1986. Germline and somatic mosaicism in transgenic mice. *Dev. Biol.* 118:9–18
90. Zhu, Z., Li, G., He, L., Chen, S. 1985. Novel gene transfer into the fertilized eggs of gold fish (*Carassius autatus* L. 1758). *Z. Angew. Ichthyol.* 1:31–34