1 Bioengineering of Farm Animals: Meat Quality and Safety

Morse B. Solomon, Janet S. Eastridge, and Ernest W. Paroczay
Food Technology and Safety Laboratory, USDA*

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A tremendous amount of variation in muscle and meat characteristics exists among and within breeds and species. Conventional science to improve muscle and meat parameters has involved breeding strategies, such as selection of dominant traits or selection of preferred traits by crossbreeding, and the use of endogenous and exogenous growth hormones. Improvements in the quality of food products that enter the market have largely been the result of postharvest intervention strategies. Biotechnology is a more extreme scientific method that offers the potential to improve the quality, yield, and safety of animal products by direct genetic manipulation of livestock. In essence, biotechnology is a new approach to the methods of genetic selection, crossbreeding, or administration of growth hormones in its final result. However, progress in this area is very slow and has a long way to go before having an impact at a commercial usage level.

Biotechnology in animals is primarily achieved by cloning, transgenesis, or transgenesis followed by cloning. Animal cloning is a method used to produce genetically identical copies of a selected animal (i.e., one that possesses high breeding value),

* Mention of brand or firm names does not constitute an endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

and transgenesis is the process of altering an animal's genome by introducing a new, foreign gene (i.e., DNA) not found in the recipient species, or deleting or modifying an endogenous gene with the ultimate goal of producing an animal expressing a beneficial function or superior attribute (e.g., adding a gene that promotes increased muscle growth). A combination of the two methods, transgenic cloning, is the process of producing a clone with donor cells that contain heritable DNA inserted by a molecular biology technique, as used in a transgenic event. A pioneering report by Palmiter et al. (1982) on the accelerated growth of transgenic mice that developed from eggs microinjected with a growth hormone fusion gene started the revolution in biotechnology of animals. Based on this research, many novel uses for biotechnology in animals were envisioned, beginning with enhancement of production-related traits (yield and composition) and expanding into disease resistance strategies and production of biological products (i.e., pharmaceuticals).

Early methods of cloning involved a technology called embryo splitting, but the traits of the resulting clones were unpredictable. Today's method of cloning, somatic (adult) cell nuclear transfer, became established in 1997 with the production of the world's first cloned farm animal, Dolly the sheep (Wilmut, Schnieke, McWhir, Kind, and Campbell 1997), and has since been used for cattle, goats, mice, and pigs. Cloning could be a promising method of restoring endangered or near-extinct species and populations. Production of transgenic animals is carried out by a technique called pronuclear microinjection, reported first in mice (Gordon, Scangos, Plotkin, Barbosa, and Ruddle 1980), and later adapted to rabbits, sheep, and pigs (Hammer et al. 1985). An excellent review on genome modification techniques and applications was published by Wells (2000).

Before 1980, applications for patents on living organisms were denied by the U.S. Patent and Trademark Office (USPTO) because anything found in nature was considered nonpatentable subject matter. However, U.S. scientist Ananda Chakrabarty, who wanted to obtain a patent for a genetically engineered bacterium that consumes oil spills, challenged the USPTO in a case that landed in the U.S. Supreme Court, which in 1980 ruled that patents could be awarded on anything that was human-made. Since then, some 436 transgenic or bioengineered animals have been patented, including 362 mice, 26 rats, 19 rabbits, 17 sheep, 24 pigs, 20 cows, 2 chickens, and 3 dogs (Kittredge 2005). Due to steps specific to transgenic procedures, for instance the DNA construct, its insertion site, and the subsequent expression of the gene construct, animals derived from transgenesis have more potential risks than cloned animals. Based on a National Academy of Sciences (NAS), National Research Council (NRC) report (2002), "Animal Biotechnology: Science-Based Concerns," the U.S. Food and Drug Administration (FDA 2003) announced that meat or dairy products from cloned animals are likely to be safe to eat, but to date has not yet approved these products for human consumption. The NAS report recommended a rigorous and comprehensive evaluation on two key issues: 1) collecting additional information about food composition to be sure that these food products are not different from normal animals, and 2) an evaluation of health status indicators of genetically engineered animals and their progeny. Even if FDA regulatory approval is granted, consumer perceptions of genetically engineered animals as food products would need to be addressed. There is a popular belief that alterations to the normal

genetic makeup triggers the creation of harmful new compounds, or that food products derived from genetically altered animals created in a laboratory are considerably less wholesome and more risky to eat compared to a normal animal raised on a farm. On the other hand, the use of biotechnology in animals to treat infectious diseases or produce new vaccines may be widely accepted. In any event, bioengineered animal products won't be on the market in the foreseeable future: High costs ($20,000–$200,000 each), extremely low efficiency rate (< 1% for livestock, < 4% for mice), and the several-year investment of time needed to generate these animals and progeny need to be overcome. The low efficiency of the process can be attributed to three factors: embryo survival, gene integration rate, and gene expression. The majority of original genetic engineering research reports focus on developing faster growing animals.

In the U.S., bioengineered foods are regulated by three agencies: the U.S. Department of Agriculture (USDA), FDA and Environmental Protection Agency (EPA). The USDA has oversight for meat and poultry, whereas seafood regulation falls under the FDA. The FDA Center for Veterinary Medicine (CVM) also regulates transgenic animals because any drug or biological material created through transgenesis is considered a drug and has to undergo the same scrutiny to demonstrate safety and effectiveness (Lewis 2001). The EPA has responsibility for pesticides that are genetically engineered into plants. In the mid-1980s, federal policy declared that biotechnologically derived products would be evaluated under the same laws and regulatory authorities used to review comparable products produced without biotechnology. As stated on the FDA Web site, the CVM has asked companies not to introduce animal clones, their progeny, or their food products into the human or animal food supply until there is sufficient scientific information available on the direct evaluation of safety.

1.1 BOVINE

Information in this area is very limited and highly desired by federal agencies that regulate food safety issues. There have been some studies evaluating the meat of animals cloned from embryonic cells (Gerken, Tatum, Morgan, and Smith 1995; Harris et al. 1997; Diles et al. 1999). Those results, however, do not correspond with products from animals cloned from adult somatic cells. This is because embryonic animal clones are produced from blastomeres of fertilized embryos at a very early stage of development, and thus embryonic clones may undergo little gene reprogramming during their development. Consequently, they would not serve well as scientific evidence for assessing the food safety risks of somatic cloned food animals. A few reports that provide data on the composition of meat and dairy products derived from adult somatic cell clones indicate that these products are equivalent to those of normal animals. The first report on the chemical composition of bovine meat arising from genetic engineering was in cloned cattle (Takahashi and Ito 2004). In meat samples derived from cloned and noncloned Japanese Black cattle at the age of 27 to 28 months, data were collected for proximate analysis (water, protein, lipids, and ash) as well as fatty acids, amino acids, and cholesterol. The results of this study showed that the nutritional properties of meat from cloned cattle are similar to those of noncloned animals, and were within recommended values of

Japanese Dietetic Information guidelines. Also, based on the marbling score, the meat quality score of the cloned cattle in this study graded high (Class 4) according to the Japanese Meat Grading Standard (ranging from Class 1 [poor] to Class 5 [premium]). No other carcass characteristics were discussed in this report.

A comprehensive study designed specifically to provide scientific data desired by U.S. regulatory agencies on the safety issue of the composition of meat and milk from animal cloning was recently published (Tian et al. 2005). All animals were subjected to the same diet and management protocols. The study analyzed more than 100 parameters that compared the composition of meat and milk from beef and dairy cattle derived from cloning to those of genetic- and breed-matched control animals from conventional reproduction. The beef cattle in this study were slaughtered at 26 months of age and also examined for meat quality and carcass composition. A cross-section between the sixth and seventh rib of the left side dressed carcass was inspected according to Japan Meat Grading Association guidelines. Additional parameters of the carcass analyzed were organ or body part weights, and total proportion of muscle and fat tissue to carcass weight. The histopathology of seven organs was examined for appearance of abnormalities. Six muscles (*Infraspinatus, Longissimus thoracis, Latissimus dorsi, Adductor, Biceps femoris*, and *Semitendinosus*) were removed from the carcass and measured for percentages of moisture, crude protein, and crude fat. Sampling from these muscles for muscle fiber type profiling, however, was not performed. The fatty acid profile of five major fat tissues (s.c. fat, intra- and intermuscular fats, celom fat, and kidney leaf fat) and the amino acid composition of the *Longissimus thoracis* muscle were also determined. Out of the more than 100 parameters examined, a significant difference was observed in 12 parameters for the paired comparisons (clone vs. genetic comparator and clone vs. breed comparator). Among these 12 parameters, 8 were related to the amount of fat or fatty acids in the meat or fat. The other four parameters found different between clones and comparators were yield score, the proportion of *Longissimus thoracis* muscle to body weight, the muscle moisture, and the amount of crude protein in the *Semitendinosus* muscle, and all fell within the normal range of industry standards. Therefore, none of these parameters would be cause for concern to product safety.

The mechanisms of regulation of muscle development, differentiation, and growth are numerous and complex. Meeting the challenge of optimizing the efficiency of muscle growth and meat quality requires a thorough understanding of these processes in the different meat-producing species. Application of biotechnology for livestock and meat production potentially will improve the economics of production, reduce environmental impact of production, improve pathogen resistance, improve meat quality and nutritional content, and allow production of novel products for the food, agricultural, and biomedical industries.

In a recent article, Wall et al. (2005) reported on the success of genetically enhanced cows with lysostaphin to resist intramammary *Staphylococcus aureus* (mastitis) infection. Mastitis is the most consequential disease in dairy cattle and costs the U.S. dairy industry billions of dollars annually. Their findings indicated that genetic engineering of animals can provide a viable tool for enhancing resistance to disease, thus improving the well-being of livestock.

1.2 OVINE

Although the first mammalian species to be cloned using a differentiated cell (Wilmut et al. 1997) was ovine, continued development of cloning technology in this species has been in support of conserving endangered species (Loi et al. 2001; Ryder 2002). About 5% to 10% of cloned sheep embryos result in offspring, but not all are healthy. Several groups have attempted transgenic introduction of growth hormone genes in sheep, but none have resulted in commercially useful transgenic animals. Growth-promoting transgenes in sheep was first accomplished by Hammer et al. (1985), followed by Rexroad et al. (1989, 1991), where gene constructs inserted into the sheep produced a 10 to 20 times elevation of plasma growth hormone level. Growth rates were similar to control sheep early in life, but after 15 to 17 weeks of life, the overexpression of growth hormone was cited by Ward et al. (1989) and Rexroad et al. (1989) to be responsible for reduced growth rate and shortened life span. Ward et al. (1990) summarized their studies with transgenic sheep, noting reduced carcass fat, elevated metabolic rate and heat production, skeletal abnormalities, and impaired survival due to the unregulated production of growth hormone in the transgenic sheep unless an all-ovine construct was used.

The pattern of expression of the various growth hormone (GH) and growth-hormone releasing factor (GRF) transgenes in sheep could not be predicted (Murray and Rexroad 1991), as circulating levels of growth hormone and IGF-I levels did not correlate to expression of the transgenes. Transgenic sheep that were nonexpressing had transgenic progeny that also failed to express the transgene (Murray and Rexroad 1991). Transgenic lambs that expressed either GH or GRF had growth rates similar to nontransgenic controls even though the transgenic lambs had elevated plasma levels of IGF-I and insulin. Early literature on transgenic sheep expressing GH indicated similar growth rates and feed efficiency (Rexroad et al. 1989) as nontransgenic controls; however, all transgenic sheep displayed pathologies and shortened life span. Further, transgenic sheep expressing GH were noted to have significantly reduced amounts of body and perirenal fat (Ward et al. 1990; Nancarrow et al. 1991) and were also susceptible to developing chronically elevated glucose and insulin levels of diabetic conditions.

Progress in overcoming the health problems of GH transgenic sheep was made by switching to an ovine GH gene with ovine metallothionein promoter (Ward and Brown 1998). They encountered no health problems through, at least, the first four years of life, although Ward and Brown (1998) noted increased organ sizes and noticeably reduced carcass fat in the G1 generation. Twenty transgenic lambs of the G2 generation (Ward and Brown 1998) grew significantly faster than controls, with differences detected between rams and ewes. Growth rate of transgenic rams was greater than controls from birth onward, whereas increased growth rate in transgenic ewes was not noted until four months of age. No difference in feed conversion from four to seven months of age was observed between control and transgenic lambs (Ward and Brown 1998). In the G3 generation, Brown and Ward (2000) reported the average difference in body weight between transgenic and controls at 12 months of age was 8% and 19% heavier for rams and ewes, respectively. Their results were

consistent with the increased circulating levels of GH in transgenics compared to controls.

Piper, Bell, Ward, and Brown (2001) evaluated the effects of an ovine GH transgene on lamb growth and wool production performance using 62 transgenic Merino sheep. The G4 transgenic lambs were from a single transgenic founder ram and were compared to 46 sibling controls. Preweaning body weights were similar for transgenic and controls, but began to diverge and were significantly different from seven months of age onward. Transgenic lambs were about 15% larger than controls at 12 months of age and had very low amounts of subcutaneous fat. Major wool production traits, greasy fleece weight and mean fiber diameter, were not different from controls.

Adams, Briegel, and Ward (2002) also examined the effects of a transgene encoding ovine GH and an ovine metallothionein promoter in progeny of 69 Merino and 49 Poll Dorset lambs from ewes inseminated by G4 transgenic rams heterozygous for the gene construct. As seen in earlier research using mouse-derived GH transgenes, the effects of the ovine construct varied according to active expression of the transgene. The transgene failed to be expressed in some progeny (Adams et al. 2002) despite positive status for the transgene. The ovine GH produced negligible health problems, similar to that reported by Ward and Brown (1998). Among progeny with active transgene expression, plasma GH levels were twice those of controls. Those sheep also grew faster to heavier weights and were leaner, but had higher parasite fecal egg counts compared to nontransgenic sheep. Females at 18 months of age had decreased *Longissimus* muscle depth compared to males. Adams et al. (2002) concluded that phenotypic effects of genetic manipulation of sheep may depend on age, breed, and sex of the animal and that modification to the fusion genes is required to meet the species-specific requirements to enhance expression in transgenic sheep while maintaining the long-term health status.

Callipyge sheep have muscle fiber hypertrophy determined by a paternally inherited polar overdominance allele (Cockett et al. 1994) that is a result of a single base change (Freking et al. 2002; Freking, Smith, and Leymaster 2004). This naturally occurring mutation that alters muscle phenotype in sheep was described by Jackson and Greene (1993) and Cockett et al. (1994), and since has been the subject of much research. The callipyge phenotype is a posttranslational effect (Charlier et al. 2001) in which the dam's normal allele suppresses synthesis of at least four proteins that form muscle tissue. The phenotype is characterized by hypertrophy in certain muscles (viz., *Longissimus thoracis et lumborum* [LTL], *Gluteus medius, Semimembranosus, Semitendinosus, Adductor, Quadriceps femoris, Biceps femoris* [BF] and *Triceps brachii*), whereas other muscles (*Infraspinatus* [IS] and *Supraspinatus* [SS]), are unaffected. The hypertrophy is caused by increased size of the fast-twitch fibers rather than increased fiber numbers (Carpenter, Rice, Cockett, and Snowder 1996). Lorenzen et al. (1997) measured an elevated protein to DNA ratio in callipyge LTL and BF but not in IS and SS. Fractional protein accretion rate did not differ among those muscles, and protein synthesis rate was decreased by 22% in callipyge LTL and by 16% in callipyge BF muscles. Because the protein degradation rate was also decreased by 35% in callipyge compared to controls, Lorenzen et al. (1997) concluded that callipyge-induced muscle hypertrophy was due to decreased muscle protein degradation.

Reduced tenderness in callipyge was also related to higher calpastatin (Goodson, Miller, and Savell 2001; Freking et al. 1999; Koohmaraie, Shackelford, Wheeler, Lonergan, and Doumit 1995) and m-calpain activities (Koohmaraie et al. 1995) compared to control sheep. Otani et al. (2004) presented evidence in mice that overexpression of calpastatin contributes to muscle hypertrophy, although this has not been investigated in relation to the callipyge phenotype.

Busboom et al. (1994) indicated that callipyge lambs had less monounsaturated and more polyunsaturated fatty acids than controls. Muscle hypertrophy in callipyge sheep was also at the expense of adipose tissue (Rule, Moss, Snowder, and Cockett 2002), possibly from a decrease in differentiation of adipocytes. Rule et al. (2002) measured lower lipogenic enzyme activities in adipose tissues of heterozygous callipyge lambs compared to controls but were unable to relate these differences to insulin or IGF-I levels. The callipyge locus has been mapped to a chromosome segment that carries four genes that are preferentially expressed in skeletal muscle and are subject to parental imprinting, namely, Delta-like 1 (DLK1), gene-trap locus 2 (GTL2), paternal expressed gene 11 (PEG11), and maternal expressed gene 8 (MEG8). The same conserved order was found on human and mouse chromosomes. The causative mutation for callipyge is a single base transition from A to G in the intergene region between DLK1 and GLT2 (Bidwell et al. 2004). Charlier et al. (2001) demonstrated the unique very abundant expression of DLK1 (involved in adipogenesis) and PEG11 (unknown function) in callipyge sheep; however, they were not able to explain how the overexpression of these genes was related to muscle hypertrophy. They suggested that the callipyge mutation does not alter the imprinting of DLK1 or PEG11, but modifies the activity of a common regulatory element that could be an enhancer or silencer. Bidwell et al. (2004) similarly detected elevated DLK1 and PEG11 in muscles of lambs with the callipyge allele and named them as candidate genes responsible for the skeletal muscle hypertrophy. PEG11 was 200 times higher in heterozygous and 13 times higher in homozygous callipyge sheep than in controls. Freking et al. (2004) discussed expression profiles and imprint status of genes near the mutated region of the callipyge locus. Markers for polymorphic genes that control fat and lean, such as thyroglobulin, or the callipyge gene, could be used for making genetic selection improvements in animals (Sillence 2004).

The apparent advantages of higher carcass yield, increased lean, and reduced fat content of callipyge sheep would benefit the meat industry except for the associated toughness in the hypertrophied muscles. In contrast to minimal tenderness improvement using antemortem techniques to control growth rate, size, or fatness level (Duckett, Snowder, and Cockett 2000) or treatment with dietary vitamin D_3 (Wiegand, Parrish, Morrical, and Huff-Lonergan 2001), some success at improving tenderness of meat from callipyge has been accomplished by various postmortem treatments. Tenderness was improved slightly by electrical stimulation (Kerth, Cain, Jackson, Ramsey, and Miller 1999). Other postmortem treatments effective for improving tenderness in callipyge include prerigor freezing prior to aging (Duckett, Klein, Dodson, and Snowder 1998), calcium chloride injection (Koohmaraie, Shackelford, and Wheeler 1998), hydrodynamic pressure treatment (Solomon 1999), and extended aging to 48 days (Kuber et al. 2003). The higher calpastatin level responsible for the hypertrophy of callipyge lambs (Freking et al. 1999; Goodson et al. 2001;

Koohmaraie et al. 1995) is often cited as contributing to the lower tenderness of the meat because calpastatin interferes with the normal postmortem proteolysis during aging, particularly the breakdown of troponin-T (Wiegand et al. 2001). The lack of tenderness associated with the callipyge gene must be addressed before the economic advantages can be realized.

1.3 CAPRINE

Prior to the first transgenic goat, Fehilly, Willadsen, and Tucker (1984) produced an interspecies chimera between sheep and goat, the geep. Today, cloning (Behboodi et al. 2004) and embryo splitting (Oppenheim, Moyer, Bondurant, Rowe, and Anderson 2000) are employed as the most rapid means of highly focused initial expansion of a transgenic herd. This approach combines the two techniques by first creating the transgenic goat with the desired traits. Cloning is then used to create replicas of the transgenic animal. Goats have cloning efficiency of 3% to 7%. The benefits of cloned and transgenic goats are accelerated genetic improvements in production of hair, meat, and milk; however, the production of products in goat milk for the pharmaceutical industry is the most widely used application of this technology.

Goats, rabbits, and flies are often employed for recombinant protein production because mice do not efficiently scale up, transgenic cattle take too long to prepare, plants produce pollen that drifts in the wind, and chickens have problems with long-term stability of germ-line expression as well as carrying viruses and new strains of flu (Anonymous 2004). Goats, then, are the animal of choice for biomedical and industrial bioreactors for the production of protein therapeutics for the health care and agro-biotech industries (Baldassarre, Wang, Keefer, Lazaris, and Karatzas 2004; Goldman, Kadulin, and Razin 2002; Ko et al. 2000; Nicholls 2004; Tulsi 2004). Transgenic goats require much less capital investment, are more efficient than manufacturing systems using cell culture (Tulsi 2004), and are easier to scale up production. Published literature lacks information regarding the amount of hair, milk, or meat produced using transgenic goats. The products produced through transgenic goats primarily are pharmaceutical and are regulated by the FDA.

1.4 PORCINE

Among major livestock species, the pig was last to be cloned (Betthauser et al. 2000; Onishi et al. 2000; Polejaeva et al. 2000). There appears to be more interest in transgenesis and cloning of pigs as a model for studying human diseases, such as osteoporosis and diabetes, and for donor organs for xenotransplantation rather than for improving meat production. Pigs, due to their vast numbers and similar organ size and function to humans, are desirable for xenotransplantation. Hyperacute rejection of xenotransplanted organs was a major concern until Prather, Hawley, Carter, Lai, and Greenstein (2003) accomplished genetic modification of the $\alpha(1,3)$-galactosyltransferase gene prior to nuclear transfer cloning. Nuclear transfer cloning efficiency rates for swine average between 1% and 6% of embryos. This and other issues need to be solved with this technology. Cloned pigs appear to have inadequate

immune systems (Carroll, Carter, Korte, Dowd, and Prather 2004), display behavioral variations (Archer, Friend, Piedrahita, Nevill, and Walker 2003), and could transmit viruses (van der Laan et al. 2000). In contrast, Carter et al. (2002) used green fluorescent protein transgene then cloned pigs to evaluate phenotype and health status. They declared that cloned pigs can be normal and without impaired immune systems.

Approximately 40% of the red meat consumed worldwide comes from pigs (Food and Agriculture Organization of the United Nations 2004), and pork consumption has increased consistently with increasing world population. Continued improvements in pork production, therefore, are needed to meet future demands for red meat. Research in genomics is one avenue to increase production efficiency. Selection of pigs based on the ryanodyne receptor (RyR) gene, muscle regulatory factor (MRF) gene family, hormones, or other potential candidate genes affecting growth and fattening traits is needed to increase production. Quantitative trait loci (QTL) evaluation of factors associated with meat quality and growth are underway; however, in pigs, some quality traits are polygenic (Krzecio, Kocwin-Podsiada, et al. 2004), requiring evaluation of their interactions.

QTL analysis of factors affecting tenderness and juiciness of pork were mapped to chromosome 2, and based on that location, the calpastatin (CAST) gene was considered a likely candidate (Ciobanu et al. 2004). One of three CAST haplotypes identified using a restriction enzyme (viz., RasI) was found to be associated with the investigated traits and might serve as a marker for selection and breeding. Meat quality traits in pigs negative for the halothane sensitivity ryanodyne receptor (RyR1) and RN- alleles were evaluated for interactions with CAST (Krzecio, Kury, Kocwin-Podsiada, and Monin 2004). For stress-resistant RyR1 pigs, CAST polymorphisms using RsaI restriction enzyme (CAST/RsaI) were identified as AA, AB, and BB genotypes. These were found to affect water holding capacity (WHC), drip loss, and water and protein content of muscle. CAST/RsaI AA genotype pigs had lower WHC, lower drip loss at 96 hours, less moisture, and higher protein content in muscle compared to the BB genotype. Stress-resistant pigs (homozygous and heterozygous RyR1 resistant genotype) had highly significant lactate level measured by pH at 35 and 45 minutes postmortem and on reflectance values. Homozygous stress-resistant pigs produced the most desirable quality traits. The interaction of CAST/RsaI and RyR1 was significant for *Longissimus lumborum* muscle pH at 45 minutes postmortem and drip loss at 48 hours; however, no interactions were detected for carcass lean (Krzecio, Kocwin-Podsiada, et al. 2004; Krzecio, Kury, et al. 2004) or cooking yield. That CAST and RyR1 would interact is not surprising because calpastatin is an endogenous inhibitor of calcium-dependent cysteine proteases, the calpains, and a mutation in RyR1 is partly responsible for disturbed regulation of intracellular Ca^{2+} in pig skeletal muscle (Kuryl, Krzecio, Kocwin-Podsiadla and Monin 2004). These studies indicate that quality of meat should be considered not only by each individual genotype, but also by interactions with other genes.

Polymorphisms of the CAST gene and their association between genotypes at the porcine locus myostatin (MSTN) growth differentiation factor 8 were considered by Klosowska et al. (2005). Mutations in the MSTN gene are responsible for extreme muscle hypertrophy, or double muscling, in several breeds of cattle. Myostatin is important for controlling development of muscle fibers and is considered a negative

regulator of muscle growth (McPherron, Lawler, and Lee 1997). Because calpain activity is required for myoblast fusion and cell proliferation and growth, it might also affect the number of skeletal muscle fibers. The fusion of myoblasts to form fibers is accompanied by a dramatic change in the calpain/calpastatin ratio. Overexpression of calpastatin, an endogenous calpain inhibitor, in transgenic mice resulted in substantially increased muscle tissue (Otani et al. 2004). Klosowska et al. (2005) analyzed the interaction of MSTN and CAST in Piétrain × (Polish Large White × Polish Landrace) crossbred pigs and the Stamboek line of Dutch Large White × Dutch Landrace pigs. The MSTN genotypes identified using the TaqI restriction enzyme were CC or CT, and CAST/RsaI genotypes were identified as EE, EF, or FF. Klosowska et al. (2005) reported that 79.5% of the Stamboek line was characterized as MSTN/TaqI CC genotype. Interestingly, the FF genotype of CAST/RsaI was not detected in the Piétrain crossbred pigs. Muscle fiber size and type distributions were not affected by the MSTN genotypes although there were breed differences. Piétrain crosses had larger mean fiber diameters in all fiber types compared to Stamboek pigs. Proportion of fiber types in a bundle was higher for slow-twitch oxidative (SO) and lower for fast-twitch glycolytic (FG) fibers in Piétrain crossbred pigs compared to Stamboek pigs. Of multiple deletions or substitutions identified for MSTN, only one results in muscle hypertrophy seen in double muscle cattle and in mice. The C to T replacement in the MSTN gene does not result in an amino acid substitution (Stratil and Kopecny 1999), thus, it is probable that this genotype has no effect on the myostatin function in pigs. Muscle fiber diameters and number of fibers per unit area were not different for CAST genotypes in Piétrain cross pigs, whereas the CAST genotype had an effect in the Stamboek line. In all fiber types, fiber diameters were larger in the CAST EE and EF genotypes and smallest in FF. Loin eye area of EE genotype also was significantly larger than for EF or FF genotypes. Because of the missing FF genotype in Piétrain cross pigs, the interaction of CAST and MSTN could not be assessed.

The peroxisome proliferator-activated receptor-gamma coactivator-1 (PPARGC1 or PGC-1α) gene was investigated by Kunej et al. (2005) as a potential candidate gene affecting fattening traits and pork meat quality. This gene has a single nucleotide substitution at position 1378 within the central region of PGC-1α on chromosome 8 and occurs predominantly in Western pig breeds, whereas the conserved gene occurred in 92.6% (± 4.8%) in Chinese pig breeds. These findings were associated with marked differences in fat and lean tissue depositions in Western and Chinese pig breeds. Bayesian analysis indicated that these two groups of pigs had diverged at this locus during genetic evolution of breeds. PGC-1α is a transcriptional coactivator of many nuclear hormone receptors involved in lipid metabolism and adipocyte differentiation. In humans, PGC-1α is associated with abdominal and subcutaneous fat, and PGC-1α is expressed in skeletal muscle to a greater extent in lean than in obese individuals. It can be increased in skeletal muscle by calorie restriction. Insulin-sensitive glucose transporter (GLUT4; also called SLC2A4) also is regulated by PGC-1α and was investigated as a candidate gene for meat quality traits by Grindflek, Holzbauer, Plastow, and Rothschild (2002). GLUT4 is located on porcine chromosome 12 and plays a role in muscle and adipose tissue glucose metabolism and has unique muscle and fat expression.

In transgenic mice overexpressing calpastatin, fat content was greatly reduced and GLUT4 concentration was elevated more than three times (Otani et al. 2004). Otani et al. (2004) suggested that because calpain can degrade GLUT4, inhibition of calpain also diminished GLUT4 degradation, resulting in increased muscle growth. Grindflek et al. (2002) utilized approximately 1,700 pigs from U.S. and Norwegian commercial pig lines to determine any association of GLUT4 to meat quality. Significant associations were found for GLUT4 and drip loss, marbling, and loin depth in some U.S. lines, although association of GLUT4 polymorphisms to quality traits were not consistent across lines. No significant associations were detected for any meat quality traits in the Norwegian pig population. Among reasons given for the weak associations, Grindflek et al. (2002) suggested that linkage disequilibria or interactions with other genes might cause interference.

The transgenic Enviro™ pig was created (Forsberg 2002) to be better able to digest cereal grains by utilizing the enzyme phytase. Transgenic pigs producing phytase in their saliva (Golovan et al. 2001) were able to digest 90% to 100% of the phosphorus in their diets compared to 50% in control pigs. This transgenesis would eliminate the need to supplement pig diets with phosphorus and would reduce the amount of phosphorus in their manure by about 60%. This translates to greatly reduced phosphorus concentration in manure, which would have a positive environmental impact. Phytase can be added to pig feed, but ultimately, the transgenic pig could be more cost-effective, according to Forsberg (2002). In anticipation of marketing meat from the Enviro pig, the Medical and Related Links to Agricultural Network for Development and Innovation with Guelph (MaRS LANDING) consortium in Guelph, Canada had performed extensive analysis of the meat and found it to be indistinguishable from ordinary pork (Dove 2005). Similar efforts to improve the digestibility of feeds, and hence, feed efficiency, are underway in poultry and aquaculture. Dietary cellulose and xylan digestion in poultry is by microbial fermentation in the hind gut, a relatively inefficient process. Transgenesis to express bacterial cellulase enzymes in poultry and aquaculture species could improve digestion of plant polysaccharides, increasing feed efficiency similar to that demonstrated in the mouse (Hall et al. 1993).

Transgenic pigs expressing a plant gene, spinach desaturase, for the synthesis of essential polyunsaturated fatty acids (PUFA), linoleic and linolenic acids, have been produced (Saeki et al. 2004), marking the first time that a plant gene has been functionally expressed in mammalian tissue. This transgenesis could result in significant improvement in pork quality beneficial to human health. Saeki et al. (2004) detected levels of linoleic acid in adipocytes about 10 times higher in transgenic than in control pigs. Niemann (2004) suggested that modifying the fatty acid composition of products from domestic animals might make this technology more appealing to the public. High levels of dietary PUFA were shown to improve processing and increase PUFA in pork muscle. Earlier work with transgenic pigs and with injected porcine somatotropin also led to reduced levels of saturated fatty acids in pork (Pursel and Solomon 1993; Solomon, Pursel, and Mitchell 2002).

Many reports have documented the effects on growth of pigs receiving additional GH by exogenous administration or endogenously through transgenesis (Pursel et al. 1988; Pursel and Rexroad 1993; Pursel et al. 1997; Solomon, Pursel, Paroczay, and Bolt 1994; Vize et al. 1998; Wieghart et al. 1988). Transgenic pigs expressing

IGF-I, a regulator of growth hormone, have been described in detail (Mitchell and Pursel 2003; Pursel et al. 2004; Pursel, Mitchell, Wall, Coleman, and Schwartz 2001; Pursel, Mitchell, Wall, Solomon, et al. 2001; Solomon et al. 2002). Pursel et al. (2004) summarized the advances made in pigs expressing a skeletal α-actin-hIGF-I transgene; namely, the expression of IGF-I in skeletal muscles gradually improved body composition in transgenic pigs without major effects on growth performance. Lean tissue accretion rates were significantly higher (30.3% and 31.6%), and fat accretion rates were 20.7% and 23.7% lower in transgenic gilts and boars, respectively, compared to controls. Body fat, bone, and lean tissue measurements by dual-energy X-ray absorptiometry confirmed that transgenic pigs had less fat and bone but higher lean tissue amount than control pigs.

Dietary conjugated linolenic acid (CLA) and IGF-I transgene (TG) had little or no effect on pork quality (Solomon et al. 2002; Eastridge, Solomon, Pursel, Mitchell, and Arguello 2001). Carcass weight of IGF-I TG pigs was less than non-TG controls; however, TG pigs had a 16% larger loin eye area, 26% to 28% reduced backfat thickness, and 21% less carcass fat. Dietary CLA acted synergistically with the IGF-I TG in reducing backfat thickness. Muscle pH at 45 minutes (pH_{45}) was lower ($p < .01$) in TG than non-TG (6.0 vs. 6.1) pigs, and dietary CLA resulted in significantly higher pH_{45} than for pigs fed control diets (6.1 vs. 6.0). At 24 hours, muscle pH was not different, averaging pH 5.6 for all carcasses. Neither gene status nor dietary CLA affected drip/purge loss during 21-day refrigerated storage in vacuum package, pork chop cooking yield, or thiobarbituric reactive substances measured in vacuum-packaged loins stored for 5 days and 21 days fresh and 6 months frozen. In pigs receiving the control diet, pork chop tenderness was improved significantly (i.e., lower shear force values) in IGF-I TG compared to non-TG (5.3 vs. 7.0 kgf) pigs. Dietary CLA improved tenderness in non-TG pigs equivalent to tenderness of TG pigs. Wiegand, Parrish, Swan, Larsen, and Baas (2001) detected no effects of CLA supplementation of swine diets on sensory attributes, although, it improved meat color, marbling, and firmness. Bee (2001) detected no effect of CLA on pig growth performance, carcass lean, or fat deposition, but there was a marked effect on fatty acid profiles. Saturated fatty acids, palmitic and stearic, were increased significantly, whereas monounsaturated linoleic and polyunsaturated arachidonic acids were reduced. Activity of lipogenic enzymes *in vitro* was not altered by the dietary CLA suggesting that lipogenesis was not affected by CLA (Bee 2001).

The shelf life of pork loin samples from IGF-I TG pigs with or without dietary CLA was not different from non-TG pigs (Nedoluha, Solomon, Pursel, and Mitchell 2001a). Aerobic plate counts of TG pork samples stored in retail or vacuum packages were similar to non-TG samples throughout 21 days of refrigerated storage. Ground pork from IGF-I TG pigs, with or without dietary CLA, that was inoculated with *Listeria innocua*, a nonpathogenic bacteria used as a model for *L. monocytogenes*, *E. coli* O157:H7, *Salmonella typhimurium*, and *Yersinia enterocolitica* and stored for 14 days at 7°C showed that meat from IGF-I TG pigs may be less supportive of growth of foodborne pathogens than non-TG meat (Nedoluha, Solomon, Pursel, and Mitchell 2001b). Growth of *L. innocua, E. coli, S. typhimurium,* and *Y. enterocolitica* was lower in meat from TG compared to non-TG pigs. There was no effect of dietary CLA on *Y. enterocolitica* and *E. coli*; however, *L. innocua* and *S. typhimurium* growth

was slightly higher in meat from pigs receiving CLA. More studies are needed to confirm these results.

Directing IGF-I expression specifically to skeletal muscle appeared to overcome the problems encountered with GH transgenics or with daily injections of exogenous IGF-I (Pursel et al. 2004) and clearly had a major impact on carcass composition. Piétrain pigs have 5% to 10% more meat than comparable pigs of other breeds (Houba and te Pas 2004), although, the muscle hypertrophy phenotype in Piétrain pigs is not as strongly expressed as the double-muscle condition in cattle or callipyge in sheep. The mechanism of Piétrain pig hypertrophy is still unknown; however, it might be associated with changes to the calpastatin gene. Klosowska et al. (2005) did not detect a calpastatin (CAST) polymorphism FF genotype in Piétrain cross-bred pigs. Pigs with the FF CAST genotype had smaller muscle fiber diameters compared to the EE and EF phenotypes. Linking the CAST genotype with phenotype to meat quality would benefit the meat industry, especially in pigs. The relationship between genotype at the CAST and MSTN loci to phenotype remains to be elucidated.

1.5 FOOD SAFETY IMPLICATIONS

The NRC (2002), at the request of the FDA, conducted an independent evaluation of foods from cloned animals and concluded that meat from clones and other products was safe. Based on these findings, the FDA (2003) announced that it would consider two issues: Are the animals themselves healthy, and are the products nutritionally indistinguishable from those produced by noncloned animals? After evaluating more than 100 parameters for meat and milk composition, U.S. and Japanese researchers (Tian et al. 2005) declared there were no statistical differences in these products from two Japanese Black beef and four Holstein dairy cattle clones compared to matched controls (20 beef and four dairy cattle). Walsh and Norman (2004) and Norman and Walsh (2004) also reported no differences in composition of milk from cloned cows. Few data are available on the consequence of consuming products from cloned animals. Guillén et al. (1999) evaluated consumption of transgenic tilapia by healthy human volunteers over 5 days. No differences in clinical or biochemical parameters measured were detected between those who consumed the transgenic and nontransgenic fish. Guillén et al. (1999) suggested that GH would be degraded under the ordinary acidic and enzymatic conditions during digestion in the human stomach, thus posing no effect due to consumption of the transgenic fish. Tomé, Dubarry, and Fromentin (2004) presented data from a preliminary 3-week study in which rats were fed cow's milk and meat from cloned animals. No differences between the control and cloned products were detected for food intake, body weight gain, body composition, and fasting insulin at the end of 3 weeks. Specific antimilk and meat protein immunoglobulin subtype analysis also revealed no differences between control and cloned-animal-derived products. There appeared to be no major difference in the nutritional value of milk and meat from cloned animals compared to controls. Tomé et al. (2004) cautioned that it might require a longer consumption time to confirm these observations. Technically, the introduction of novel proteins in genetically modified foods could elicit an allergic reaction (Poulsen 2004); however, there is no single test to predict allergenicity. In pigs fed transgenic plant protein

in the form of Roundup Ready soybean meal, Jennings et al. (2003) could not detect any fragment of the transgenic plant DNA nor fragment of the transgenic protein in the muscle tissue.

To date, livestock producers have honored a voluntary prohibition on requesting approval for bioengineered meat products in the United States. CBS News (2003) reported that a livestock company has made a request to Health Canada to sell meat from cloned animals but that Health Canada was still exploring the risks associated with cloned animals. The Japanese Ministry of Health, Labour and Welfare (Betterhumans 2003) concluded after a 3-year study that meat and milk products from cloned animals are safe for humans. At least 40 Japanese facilities raise cloned cattle but are prohibited under a voluntary ban from marketing the meat and milk.

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