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Transgenic alteration of sow milk to improve piglet growth and health

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There are many potential applications of transgenic methodologies for developing new and improved strains of livestock. One practical application of transgenic technology in pig production is to improve milk production or composition. The first week after parturition is the period of greatest loss for pig producers, with highest morbidity and mortality attributed to malnutrition and scours. Despite the benefits to be gained by improving lactation performance, little progress has been made in this area through genetic selection or nutrition. Transgenic technology provides an important tool for addressing the problem of low milk production and its detrimental impact on pig production. Transgenic pigs over-expressing the milk protein bovine α-lactalbumin were developed. α-Lactalbumin was selected for its role in lactose synthesis and regulation of milk volume. Sows hemizygous for the transgene produced as much as 0.9 g bovine α-lactalbumin l^{-1} pig milk. The outcomes assessed were milk composition, milk yield and piglet growth. First parity α-lactalbumin gilts had higher milk lactose content in early lactation and 20–50% greater milk yield on days 3–9 of lactation than did non-transgenic gilts. Weight gain of piglets suckling α-lactalbumin gilts was greater (days 7–21 after parturition) than that of control piglets. Thus, transgenic over-expression of milk proteins may provide a means for improving the lactation performance of pigs.

Introduction

The insertion of DNA into livestock and its stable integration into the germline have been major technical advances in agriculture. Production of transgenic livestock provides a method for introducing 'new' genes rapidly into cattle, pigs, sheep and goats without crossbreeding (Pursel and Rexroad, 1993). It is an extreme methodology but, in essence, its result is not really different from crossbreeding or genetic selection. Two basic strategies are used when producing transgenic animals. These are the so-called 'gain of function' or 'loss of function' transgenics. The basic idea behind the 'gain of function' paradigm is that by adding a cloned fragment of DNA to the genome of an animal, several aims can be achieved. One aim is to obtain new expression of a gene product that did not exist previously in that cell or tissue type. An example of this is the expression of human growth hormone (hGH) in mouse liver (Palmiter *et al.*, 1982).

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The 'loss of function' paradigm has many similar applications to the 'gain of function' strategy, especially when considering over-expression, insertional mutations and antisense situations. The major difference is the ability to disrupt genes in a targeted fashion. This strategy relies on the ability of embryonic cells to undergo homologous recombination. The generation of transgenic animals is dependent on the ability of the cell to form stable recombinants between the exogenous DNA and the endogenous chromosomal DNA in the genome of the host. Most of these events are non-homologous recombinations, in which the DNA inserts are introduced randomly into the genome. However, some cells possess the enzymatic machinery required for recombination between the introduced DNA sequence and the homologous or identical sequence in the genome of the host. This is called homologous recombination, which is often referred to as 'gene targeting'. Gene targeting permits the transfer of genetic alterations created *in vitro* into precise sites in the embryonic or cell genome (Wheeler and Choi, 1997). If the host's cells are totipotent or pluripotent embryonic cells (embryonic stem cells, embryonic germ cells or primordial germ cells) or re-programmable somatic cells, then these homologous recombination events can be transferred to the germ line of the offspring. The use of this strategy has great potential for making specific genetic changes for use in medicine and agriculture, and for furthering our understanding of the genetic control of developmental processes.

Recent advances in transgenic technology in pigs

Microinjection of cloned DNA into the pronucleus of a fertilized ovum has been the most widely used and successful method for producing transgenic mice and livestock (Hammer *et al.*, 1985). Microinjection has produced most of the transgenic pigs obtained to date. Two recent developments will have profound impacts on the use of transgenic technology in livestock: (i) the ability to isolate and maintain embryonic and somatic cells directly from embryos, fetuses and adults *in vitro*; and (ii) the ability to use these embryonic and somatic cells as nuclei donors in nuclear transfer or 'cloning' strategies. These strategies have several distinct advantages for use in the production of transgenic livestock that cannot be attained using pronuclear injection of DNA.

The use of nuclear transfer (cloning) techniques may have the potential to increase the number of offspring from a single female into the thousands and, possibly, tens of thousands (Bondioli *et al.*, 1990). Since the cloned sheep 'Dolly' was born (Wilmut *et al.*, 1997), nuclear transfer technology has become another methodology available for the production of transgenic animals.

The nuclear transfer procedure (for review see Wheeler and Walters, 2001) uses either *in vitro* or *in vivo* oocytes as the cytoplasm donor (cytoplast). The genetic material of the cytoplast is removed (enucleation) leaving only the cytoplasm. After enucleation of the oocyte, a donor nucleus (karyoplast) is injected into the perivitelline space or into the cytoplasm of the enucleated oocyte (cytoplast). The enucleated oocyte and the donor nucleus are fused by electrofusion. Electrofusion of the cytoplast and karyoplast is highly species-dependent in terms of the duration, fusion medium, fusion medium equilibration and amplitude of the pulse required. After fusion of the donor nucleus and the enucleated oocyte, the oocyte is activated by either chemical or electrical stimulation. Successful activation initiates development to the blastocyst stage, followed by transfer into a pseudopregnant recipient.

These new methods for production of genetically identical individuals from embryonic (Campbell *et al.*, 1996; Wilmut *et al.*, 1997) and somatic (Wilmut *et al.*, 1997; Polejaeva *et al.*, 2000) cells, via nuclear transfer, should allow the rapid development of genetically identical

animals with a targeted gene insertion. These developments will enhance our ability to produce transgenic animals with genes inserted into specific sites in the genome.

Recently, the birth of the world's first two sets of cloned piglets produced using fetal fibroblasts and adult somatic cells was announced (Onishi *et al.*, 2000; Polejaeva *et al.*, 2000). This achievement will allow nuclear transfer technology to be used to produce transgenic pigs.

Mammary-specific gene expression in transgenic animals

The expression of transgenes in mammary tissue has been studied in numerous laboratories (Simons *et al.*, 1987; Clark *et al.*, 1989; Vilotte *et al.*, 1989; Bleck and Bremel, 1994; Bleck *et al.*, 1996, 1998). The 5′ flanking regions of many milk protein genes, which have a regulatory function, have been used to drive expression of foreign proteins in mammary epithelial cells of transgenic animals (Simons *et al.*, 1987; Vilotte *et al.*, 1989). Of all the bovine milk protein genes, the expression of bovine α-lactalbumin is regulated most tightly and is lactation-specific (Goodman and Schanbacher, 1991; Mao *et al.*, 1991). The unique expression pattern of the bovine α-lactalbumin gene makes its promoter and regulatory elements a useful mammary expression system in transgenic animals. In contrast to the caseins and β-lactoglobulin, the production of α-lactalbumin mRNA and protein shows a marked increase at parturition, remains high during lactation and decreases sharply during cessation of lactation and involution.

Regulatory regions of milk proteins have been linked to genes that have been expressed as transgenes in a variety of animals (pigs, sheep and goats; Clark *et al.*, 1989; Ebert *et al.*, 1991; Wall *et al.*, 1991). Levels and patterns of expression have been very similar to those observed in numerous transgenic mouse experiments. These regulatory regions have shown little or no species specificity and have even been regulated properly in species that do not express those proteins (Simons *et al.*, 1987; Wall *et al.*, 1991).

Transgenic mice have been produced using the α-lactalbumin 5′ region to drive the expression of bovine, caprine or guinea-pig α-lactalbumin transgenes in the mammary gland (Vilotte *et al.*, 1989; Mashio *et al.*, 1991; Soulier *et al.*, 1992; Bleck and Bremel, 1994). Production of exogenous α-lactalbumin in the milk of these mice ranged from undetectable concentrations to up to 3.7 mg ml^{-1} in a line of mice producing caprine α-lactalbumin. However, although a number of α-lactalbumin-expressing transgenic animals have been produced, our studies are the only experiments in which lactose and milk production have been examined in transgenic pigs over-expressing α-lactalbumin (Bleck *et al.*, 1998).

Applications for modification of milk

Practical applications of transgenics in livestock production include improved milk production and composition, increased growth rate, improved feed usage, improved carcass composition, increased disease resistance, enhanced reproductive performance, increased prolificacy, and altered cell and tissue characteristics for biomedical research (Wheeler and Choi, 1997) and manufacturing. The production of transgenic pigs with growth hormone serves as an excellent example of the value of this technology (Vise *et al.*, 1988). Transgenic alteration of milk composition has the potential to enhance the production of certain proteins or growth factors that are deficient in milk (Bremel *et al.*, 1989). The improvement of the nutrient or therapeutic value of milk may have a profound impact on survival and growth of both newborn humans and animals.

Advances in recombinant DNA technology have provided the opportunity to either change

the composition of milk or produce entirely novel proteins in milk. These changes may add value to, as well as increase the potential uses of, milk.

The improvement of livestock growth or survival through the modification of milk composition requires production of transgenic animals that: (i) produce a greater quantity of milk; (ii) produce milk of higher nutrient content; or (iii) produce milk that contains a beneficial 'nutriceutical' protein. The major nutrients in milk are protein, fat and lactose. Increasing any of these components can have an impact on the growth and health of the developing offspring. In many species such as cattle, sheep and goats, the nutrients available to the young may not be limiting. However, milk production in sows limits piglet growth and, therefore, pig production (Hartmann *et al.*, 1984). In fact, studies indicate that milk yield and total milk solids composition from the sow account for 44% of the variation in weight gain of piglets (Lewis *et al.*, 1978). Methods that increase the growth of piglets during suckling result in an increase in weaning weights, a decrease in the number of days required to reach market weight and, thus, a decrease in the amount of feed needed for the animals to reach market weight.

The high percentage in growth rate attributed to milk indicates the potential usefulness of this technology for developing piglets. An approach to increase milk production in pigs may be accomplished by alteration of milk components such as lactose, a major constituent of milk in mammary gland cells. The over-expression of lactose in the milk of pigs will increase the carbohydrate intake of the developing young, resulting in improvement of piglet growth.

Cattle, sheep and goats used for meat production might also benefit from increased milk yield or composition. In tropical climates, *Bos indicus* cattle breeds do not produce copious quantities of milk. Improvement in milk yield by as little as 2–4 litres per day may have a profound effect on weaning weights in cattle such as the Nelore breed in Brazil. Similar comparisons can be made with improving weaning weights in meat type breeds such as Texel sheep and Boer goats. This application of transgenic technology could lead to improved growth and survival of offspring.

A second mechanism by which the alteration of milk composition may affect animal growth is the addition of beneficial hormones, growth factors or bioactive factors to milk through the use of transgenic animals. It has been suggested that bioactive substances in milk possess important functions in the neonate with regard to regulation of growth, development and maturation of the gut, immune system and endocrine organs (Grosvenor *et al.*, 1993). Transgenic alteration of milk composition has the potential to enhance the production of certain proteins and growth factors that are deficient in milk (Wall *et al.*, 1991). The over-expression of a number of these proteins in milk through the use of transgenic animals may improve growth, development, health and survival of the developing offspring. Some factors that have important biological functions in neonates are obtained through milk; these factors include insulin-like growth factor I (IGF-I), epidermal growth factor (EGF), transforming growth factor α (TGF-α) and lactoferrin (Grosvenor *et al.*, 1993). Alexander and Carey (1999) have suggested that oral administration of IGF-I might also improve nutrient absorptive function.

Other properties of milk that should be considered for modification are those that affect human and animal health. Pre-formed specific antibodies can be produced in transgenic animals (Storb, 1987). It should be possible to produce antibodies in the mammary gland that are capable of preventing mastitis in cattle, sheep and goats, and MMA (mastitis-metritis-agalactia) in pigs, or antibodies that aid in the prevention of domestic animal or human diseases (Pursel and Rexroad, 1993). Another example is to increase the content of proteins that have physiological roles within the mammary gland itself, such as lysozyme (Maga *et al.*, 1995) or other anti-microbial peptides.

It is important to consider the use of transgenics to increase specific components that are already present in milk for manufacturing purposes. An example might be to increase one of the casein components in milk. This could increase the value of milk in manufacturing processes such as production of cheese or yoghurt. The physical properties of a protein could also be altered, for example glycosylation of β-casein could be increased (Choi *et al.*, 1996). This would result in increased β-casein solubility. Increasing the β-casein concentration of milk would reduce the time required for rennet coagulation and whey expulsion, thereby producing firmer curds that are important in cheese making. The deletion of phosphate groups from β-casein would result in softer cheeses. Changes in other physical properties could result in dairy foods with improved characteristics, such as better-tasting low fat cheese (Bleck *et al.*, 1995). Increasing the β-casein content would result in increased thermal stability of milk that could improve manufacturing properties, as well as the storage properties of fluid milk and milk products. Eventually, it may be possible to increase the concentration of milk components while maintaining a constant volume. This could lead to greater product yield – more protein, fat or carbohydrate from a litre of milk. This would also aid in manufacturing processes, as well as potentially decreasing the transportation costs of the more concentrated products in fluid milk. The end result would be a more saleable product for the dairy producer.

An important application of transgenic technology is the production of therapeutic proteins for human clinical use in so-called 'bio-reactors'. Through genetic engineering it has become possible to produce any protein from any animal, plant or bacterial species in the milk of mammals (Bremel *et al.*, 1989; Rudolph, 1999). For example, it is possible to express milk proteins and other proteins of pharmaceutical value in the milk of mice, rabbits, pigs, goats and sheep (Simons *et al.*, 1987; Buehler *et al.*, 1990; Ebert *et al.*, 1991; Wall *et al.*, 1991; Wright *et al.*, 1991; Velander *et al.*, 1992). Advantages of the mammary synthesis of proteins include the ability of the mammary secretory cells to modify the protein properly so it is biologically active and to then secrete the protein-containing fluid (milk) in large quantities. The ability to produce large quantities of bioactive proteins and peptides transgenically has resulted in the development of a new segment of the pharmaceutical industry, which has become known as 'bio-pharming' (Rudolph, 1999).

Genes coding for proteins with pharmaceutical value such as human blood clotting factor IX, which is genetically deficient in haemophiliacs, may be incorporated into transgenic sheep, goats and cows. This protein can be harvested from the milk, purified and provided therapeutically to serum albumin for artificial blood substitute. Hepatitis antigens could also be made available for vaccine production.

The overall result of the transgenic modification of milk will be the creation of more uses of milk and milk products in both agriculture and medicine. This truly is a 'value-added' opportunity for animal agriculture by increasing the concentrations of existing proteins or producing entirely new proteins in milk.

Improvement of lactation performance of sows

Large increases in average milk production of dairy cattle have been achieved over several decades because of intense selection for a trait that is easy to measure objectively, milk yield. However, despite the importance of milk production for fast growth of the offspring of pigs, negligible increases in milk production have been made in pigs. High milk production is particularly important given the emphasis on increasing litter size. Although research has provided more insight into the process of milk secretion, understanding of the physiological factors that control the amount of milk a mammal produces is limited. Previous work has

suggested that the volume of milk produced is directly dependent on the amount of lactose synthesized. Lactose is synthesized in the Golgi apparatus of mammary secretory cells by the lactose synthase complex (Brew and Grobler, 1992). This complex is composed of the mammary specific protein α-lactalbumin and the enzyme β1,4 galactosyltransferase. Secretory vesicles are budded off from the Golgi complex, transported to the apical membrane of the epithelial cell and secreted into the lumen. As lactose cannot diffuse out of the vesicles, it acts to draw water by osmosis into the vesicle. Lactose synthase is critical in the control of milk secretion: it is necessary for the production of lactose and the movement of water into the mammary secretory vesicles and into the lumen of the gland (Hayssen and Blackburn, 1985). There is evidence to suggest that milk volume is related directly to expression of the α-lactalbumin gene (Goodman and Schanbacher, 1991). α-Lactalbumin is a normal constituent of milk and its expression correlates with the induction of copious milk secretion at the onset of lactation (Goodman and Schanbacher, 1991).

High milk production is vital for growth of offspring. Low milk production is manifested not only by slow growth before weaning but also by slow growth later in life, as animal performance also suffers through the growing and finishing stages. Current pig production management schemes attempt to maximize the number of piglets born per litter and piglet survival (Hartmann *et al.*, 1984). In addition, pork producers have reduced the duration of lactation to maximize the number of piglets born per sow per year. Currently, 10–14 days of lactation is becoming common in the pig industry. Thus, increased milk production in early lactation must be obtained to get maximum growth from larger litter sizes and shorter lactation periods. Early weaning, gains in decreasing neonatal mortality and increased litter sizes from selected high genetic merit sows make milk production one of the most important limiting factors in piglet growth. In fact, studies indicate that milk production and milk composition of the sow account for 44% of the growth weight of the piglets (Lewis *et al.*, 1978).

The effect of increased sow milk production on US pork production is marked. Using current milk production values (Auldist *et al.*, 1998), we estimate that increasing milk production by 10% would result in an additional $2.46 per litter, which would be worth $28.4 million per year in the USA due to increased weight gains before weaning, using a typical hog price of $50 per cwt. Modern sows can produce approximately 1 kg of milk per piglet for litter sizes of ≤ 14 pigs (Auldist *et al.*, 1998). This calculation does not consider decreased feed and labour costs associated with rearing pigs with heavier weaning weights.

Results from studies of α-lactalbumin transgenic pigs have several applications in animal agriculture. Firstly, lines of breeding stock to improve milk production and piglet growth can be established as a result of increased milk production and piglet growth. Secondly, as overproduction of α-lactalbumin increases milk yield, assays for α-lactalbumin can be used as a selection method for increased milk production in sows.

Production and characterization of transgenic pigs expressing bovine α-lactalbumin

Production of transgenic pigs expressing bovine α-lactalbumin

Two lines of transgenic pigs have been produced containing the bovine α-lactalbumin gene. DNA was isolated from ear biopsies for each of the piglets. PCR was performed using two separate primer sets specific for the bovine α-lactalbumin 5′ flanking region. This transgene is inherited in a normal Mendelian fashion in F_1 crosses (Bleck *et al.*, 1996, 1998). The two lines have also been mated to produce homozygous individuals. Tissue collected from nine transgenic animals was subjected to northern blot analysis. Results showed that expression of the transgene was specific to the mammary gland and not to other tissues (brain,

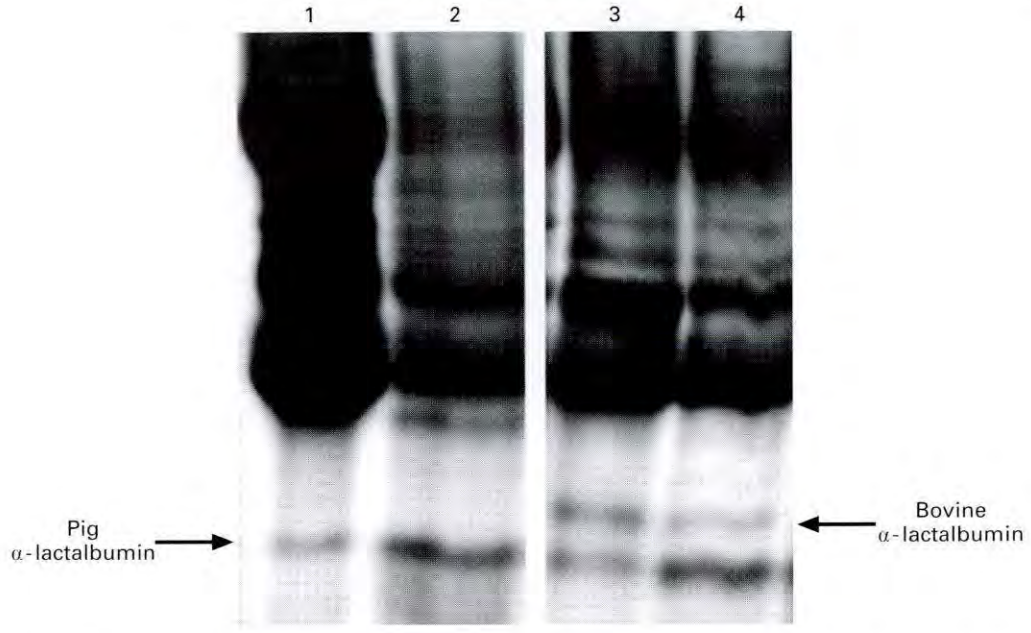

Fig. 1. Transgenic bovine α-lactalbumin is more abundant at the start of lactation than on day 5. Milk from a transgenic pig and her control full sister was separated by non-reducing urea PAGE. Lane 1: control pig milk on day 0 of lactation. Lane 2: control pig milk on day 5 of lactation. Lane 3: transgenic pig milk on day 0 of lactation. Lane 4: transgenic pig milk on day 5 of lactation.

heart, muscle, kidney, liver, skin, spleen, lung, stomach, intestine and ovary; M. B. Wheeler, M. Monaco and S. M. Donovan, unpublished).

Characterization of transgenic pigs expressing bovine α-lactalbumin

Expression of bovine α-lactalbumin in milk. Milk samples were collected from one line of transgenic pigs. In the analysis, five hemizygous transgenic gilts were matched by breed, age and farrowing season with eight control gilts of the same breed, age and farrowing season. Gilts were mated, allowed to farrow and litter size was set at ten piglets. Milk samples were collected from each animal on day 0 (after the completion of farrowing) and days 5, 10, 15 and 20 of lactation. Milk was analysed for the presence of bovine α-lactalbumin using an ELISA specific for bovine α-lactalbumin (Mao *et al.*, 1991). Transgenic sows produced bovine α-lactalbumin in their milk at concentrations ranging from 0.3 to 0.9 mg ml^{-1}. The concentration of bovine α-lactalbumin in pig milk was highest on days 0 and 5 of lactation and decreased as lactation progressed. Sows hemizygous for the transgene produced an average of 0.68 g bovine α-lactalbumin l^{-1} pig milk on day 0 of lactation (Fig. 1). The production of the bovine protein caused an approximate 50% increase in the total α-lactalbumin concentration of pig milk throughout a lactation (although the increase was dependent on the stage of lactation). The production of bovine α-lactalbumin in a single animal from day 0 to day 5 is shown (Fig. 1). The ratio of bovine to pig α-lactalbumin also appears to change during this interval. Milk from five first lactation transgenic sows was analysed to compare the relative concentrations of pig and bovine α-lactalbumin. The ratio of bovine α-lactalbumin:pig α-lactalbumin was 4.3:1.0 on day 0 of lactation, but by day 20 of

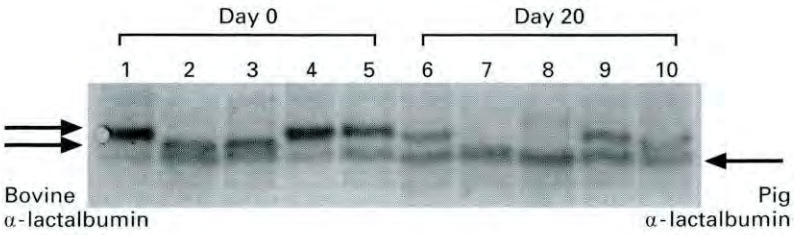

Fig. 2. Western blot of a non-reducing urea PAGE of transgenic pig milk. Milk samples were collected from five first parity transgenic sows. Lanes 1–5: milk samples from day 0 of lactation. Lanes 6–10: milk samples from day 20 of lactation.

lactation the ratio was 0.43:1.0 (Bleck *et al.*, 1998), indicating that the bovine transgene and the endogenous pig gene are under slightly different control mechanisms. These ratios were calculated by densitometry of the western blot shown (Fig. 2). A non-reducing urea PAGE system was used to separate bovine from pig α-lactalbumin (Kim and Jiménez-Flores, 1993) and bovine α-lactalbumin was found to migrate slightly slower than the pig protein (Bleck *et al.*, 1998).

Milk protein and total solids of control and transgenic pigs. No consistent significant differences were observed in the concentration of total milk protein and total solids between control and transgenic animals. Total milk protein percentage in both transgenic and control sows decreased as lactation advanced, reaching a relatively constant percentage at day 10 of lactation. This pattern is similar to that observed for the concentration of bovine α-lactalbumin, indicating that expression of the transgene is regulated in a manner analogous to that of most pig milk proteins. There was a trend for the transgenic animals to have a lower percentage of protein; however, the difference was not significant in this small sample of animals. The total solids data showed much more variation than did the protein data. Transgenic sows had a significantly higher total solids percentage than did control sows on days 10 and 20 of lactation ($P < 0.01$). However, this difference was not consistent throughout lactation (Bleck *et al.*, 1998). Recently, these results have been confirmed with pairs of full sibling (20 control and 20 transgenic) gilts (Noble *et al.*, 2000a,b; in press).

Lactose concentration of control and transgenic pig milk. The higher concentration of total α-lactalbumin present on day 0 of lactation was correlated with higher lactose percentage on day 0 in transgenic sows (3.8%) compared with controls (2.6%) ($P < 0.01$) (Fig. 3). There was also a trend for higher lactose percentage in transgenic sows on days 5 and 10 of lactation, but no significant differences were observed. Mean lactose percentage over the entire lactation period (days 0, 5, 10, 15 and 20) was also calculated. Mean lactose percentage of transgenic sows averaged 5.43%, whereas for control sows the average was 4.89%; this difference is not significant. The significant difference in mean lactose percentage on day 0 of lactation was also observed in the second lactation of these pigs. The lactose analysis for the second lactation was performed on four transgenic animals and five control animals. The lactose percentage on day 0 was 3.7% for transgenic sows and 2.6% for control sows ($P < 0.01$). These data suggest that α-lactalbumin is a limiting factor early in lactation of pigs. Furthermore, it is possible that higher concentrations of α-lactalbumin early in lactation may boost lactation, causing maximal milk output at an earlier time (Noble *et al.*, 2000b).

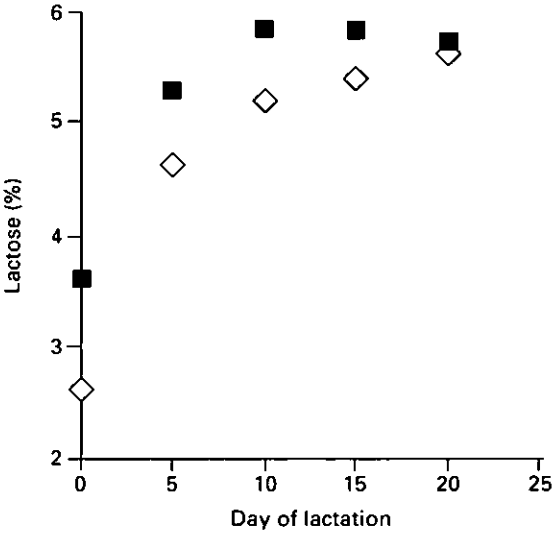

Fig. 3. Mean milk lactose percentage from first parity transgenic α-lactalbumin sows (■; n = 5) compared with control sows (◇; n = 8) over a 20 day lactation (first parity). Lactose percentage was significantly different between transgenic and control sows on day 0 of lactation ($P < 0.01$). A trend towards higher lactose percentage in the transgenic sows was also observed on days 5 and 10 of lactation; however, the difference was not significant.

Milk production in transgenic pigs

The bovine gene construct used in these experiments (see above) induced the production of α-lactalbumin at approximately 50% of normal endogenous pig α-lactalbumin expression. The bovine α-lactalbumin produced by sows appeared to be the same size as endogenous bovine α-lactalbumin. Interestingly, the concentration of bovine α-lactalbumin was highest on day 0 of lactation and decreased as lactation progressed. That pattern was similar to the trend shown for total milk protein concentration. In contrast to bovine α-lactalbumin, pig α-lactalbumin concentration was lowest on day 0 of lactation and became higher later in lactation. These data suggest that in these transgenic pigs the bovine α-lactalbumin gene was regulated differently from pig α-lactalbumin and was behaving more like other pig milk proteins.

These findings show that α-lactalbumin concentration in pig milk can be increased by the use of transgenic pigs, indicating that lactose production in early lactation can be improved. Furthermore, because of the osmoregulatory role of lactose, it is possible that higher concentrations of α-lactalbumin early in lactation may boost milk yield. We are currently examining early lactation performance in greater detail using a larger number of animals.

Recently, the milk production by and the growth of piglets suckling first parity gilts that express this bovine α-lactalbumin transgene in addition to their endogenous pig α-lactalbumin gene were examined (Noble *et al.*, 2000a). Weigh-suckle-weigh analysis was used to assess milk production from transgenic (n = 16) and control (n = 20) gilts on days 3, 6, 9 and 12 of lactation. Overall, milk production in transgenic gilts was greater than in controls

($P < 0.01$). Milk yields for transgenic gilts were 5.18 ± 0.14, 7.33 ± 0.19 and 7.40 ± 0.22 kg day^{-1} on days 3, 6 and 9, respectively. Milk yields for the controls were 4.33 ± 0.13, 5.86 ± 0.17 and 6.68 ± 0.19 kg day^{-1} on the corresponding days of lactation. By day 12 of lactation, milk yields from transgenic gilts were similar to those of their full-sibling controls (7.21 ± 0.22 kg day^{-1} for transgenic gilts and 6.94 ± 0.2 kg day^{-1} for control gilts). Daily weights of piglets reared on transgenic and control gilts were used to assess piglet growth throughout lactation. The weight gain of piglets suckling transgenic gilts was significantly greater ($P < 0.05$) than the weight gain of control reared piglets throughout the 21 days of lactation. These results indicate that milk production is increased in early lactation by the expression of the bovine α-lactalbumin transgene in first parity gilts and that this increased milk production results in enhanced piglet growth rates.

Conclusion

These results demonstrate that the bovine α-lactalbumin gene can be expressed in the pig mammary gland and that the protein can be secreted subsequently into milk. Animals containing the transgene showed no obvious abnormal phenotype. The transgenic animals grew at the same rate as controls, reached puberty at the same time, farrowed normally, lactated normally, and their litters grew at rates consistent with or faster than controls. This is different from transgenic pigs that expressed the mouse whey acidic protein gene. In these sows, high production of the transgene resulted in poor lactational performance and agalactia in some animals (Shamay *et al.*, 1991; Wall *et al.*, 1991).

Producers have continued to reduce the duration of lactation in an attempt to maximize the number of piglets born per sow per year. This production system creates a need for sows that produce more milk in early lactation to obtain maximal piglet growth during the short lactation period. This is difficult to do in sows, as maximum milk production does not normally occur until days 21–28 of lactation (Hartmann *et al.*, 1984). In addition, the number of piglets born per litter has increased in recent years, thereby also adding to the demand for higher milk production. This study indicates that lactose production in early lactation may be increased through the over-expression of α-lactalbumin in the mammary gland. Higher lactose concentrations in early lactation would provide the developing piglet with greater energy intake leading to faster growth. Furthermore, owing to the role of lactose as the major constituent in milk, increased lactose concentrations may be associated with greater milk production at the start of lactation.

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