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Transgenic Alteration of Sow Milk

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ABSTRACT

High production of milk and its components are necessary to allow maximal growth of developing piglets. In this study, transgenic pigs were produced containing the α-lactalbumin gene, whose product is a potential limiting component in the production of milk. Two lines of transgenic pigs were produced to analyze the effects that overproduction of the milk protein α-lactalbumin may have on milk production and piglet growth. Transgenic pigs were produced through microinjection of the bovine α-lactalbumin gene. The gene construct contained 2.0 kb of 5' flanking region, the 2.0 kb coding region and 329 bp of 3' flanking region. Sows hemizygous for the transgene produced as much as 0.9 g of bovine α-lactalbumin per liter of pig milk. The production of the bovine protein caused approximately a 50% increase in the total α-lactalbumin concentration in pig milk throughout lactation. The concentration of bovine α-lactalbumin was highest on day 0 and 5 of lactation and decreased as lactation progressed. The ratio of bovine to porcine α-lactalbumin changed during the sow's lactation. This ratio was 4.3 to 1 on day 0 of lactation, but by day 20 of lactation the ratio was 0.43 to 1. This suggested that the bovine transgene and the endogenous porcine gene were under slightly different control mechanisms. The higher level of total α-lactalbumin present on day 0 of lactation was correlated with higher lactose percentage on day 0 in transgenic sows (3.8%) as compared to controls (2.6%) ($P<0.01$). Although there was also a trend for higher lactose percentage in transgenic sows on day 5 and 10 of lactation, no significant differences were observed. These data suggest that α-lactalbumin is limiting early in lactation of swine. Furthermore, higher concentrations of α-lactalbumin early in lactation may boost milk output.

(Key words : Transgenic swine, Milk proteins, Lactose, Lactose synthase)

Ⅰ. INTRODUCTION

Large increases in average milk production of dairy cattle have been realized over the past several decades because of intense selection for a trait that is easy to objectively measure, that trait being milk

1. Inadequacies of Sow Lactation

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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 swine. With the emphasis on increasing litter size, high milk production is particularly important. Although research has provided more insight into the process of milk secretion, we have only a limited understanding of the physiological factors that control the amount of milk a mammal produces. Previous work has suggested that the volume of milk produced is directly dependent upon the amount of lactose synthesized. Lactose is synthesized in the Golgi apparatus of mammary secretory cells by the lactose synthase complex. This complex is composed of the mammary specific protein α-lactalbumin and the enzyme β1,4 galactosyltransferase.

Lactose is formed inside the secretory vesicles of the mammary Golgi (Brew and Grobler, 1992). These vesicles are budded off from the Golgi complex, transported to the apical membrane of the epithelial cell and secreted into the lumen. Because lactose cannot diffuse out of the vesicles, it acts to draw water by osmosis into the vesicle. Since lactose synthase is necessary for the production of lactose and the movement of water into the mammary secretory vesicles and then into the lumen of the gland, it is critical in the control of milk secretion (Hayssen and Blackburn, 1985). There is evidence, which suggests that milk volume is directly related to the expression of the α-lactalbumin gene. 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).

The importance of this work is not just to answer an important physiological question, but this work also has the potential to increase lactose and milk production in important domestic species. High milk production is vital for growth of the offspring. Low milk production is manifested not only by slow growth before weaning but also by slow growth later in life, since animal performance also suffers through the grower and finishing stages. Current U.S. swine 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 continuously reduced lactation lengths in order to maximize the number of piglets born per sow per year. Currently in the swine industry, 10～14 day lactation periods are becoming common. In order to get maximum growth from larger litter sizes and shorter lactations, increased milk production in early lactation must be obtained. Since maximum milk production in pigs does not occur until day 21～28 of lactation, a shift in the sow lactation curve is needed to obtain this maximal piglet growth. Early weaning, decreased neonatal mortality and increased litter sizes from 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 accounts for 44% of the growth weight of the piglets (Lewis et al., 1978).

Lactation efficiency may ultimately affect sow longevity in the herd because a number of sows are culled from herds due to poor lactation perfo-rmance. Current swine management schemes attempt to maximize the number of piglets born per litter and piglet survival. In order to utilize the larger litter sizes and keep the piglets alive and healthy, maximum milk production must be obtained. Indeed, the gains that have been made in decreasing newborn mortality combined with the increased litter sizes from selected high genetic merit sows make milk production and milk composition one of the most important limiting factors in piglet survivability and growth.

Previous studies have shown that increasing milk production through the use of recombinant porcine somatotropin results in increased growth of the piglets (Harkins et al., 1989). However, this increase in milk production and growth of the piglets is not observed until day 28 of lactation. Since most swine production systems wean piglets at 10~21 days of age the potential of this technology is low. In the following studies we have attempted to increase milk production on days 1 to 21 of lactation, thus making the technology more applicable to producers needs.

The effect of increasing sow milk production to U.S. pork production is dramatic. Using current milk production values (Auldist et al., 1998) we estimate that increasing milk production 10% would result in an additional $2.46 per litter which would be worth $28.4 million/year in the U.S. due to increased weight gains prior to weaning using a typical hog price of $50/cwt. Modern sows are able to produce about 1kg of milk/piglet for litter sizes up to 14 pigs (Auldist et al., 1998). This does not consider decreased feed and labor costs associated with rearing pigs with heavier weaning weights.

Results from these studies have several applications in animal agriculture. First, if an increase in milk production or piglet growth in one of the lines of transgenic pigs occurs, lines of breeding stock to improve milk production and piglet growth could be established. Second, if overproduction of α-lactalbumin increases milk yield, assays for α-lactalbumin can be used as a selection method for increased milk production.

2. Mammary-Specific Gene Expression in Transgenic Animals

Experiments described in this review make extensive use of expression of transgenes specifically in mammary tissue, a procedure that is routinely being performed by several laboratories, including our own (Simons et al., 1987; Vilotte et al., 1989; Bleck and Bremel, 1994; Bleck et al., 1996; Bleck et al., 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 the most tightly regulated and lactation specific (Goodman and Schanbacher, 1991; Mao et al., 1991). The unique expression 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 dramatic rise at parturition, remains elevated during lactation and drops sharply during lactation cessation and involution.

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 levels up to 3.7 mg/ml in a line of mice producing caprine α-lactalbumin. Even though a number of α-lactalbumin expressing transgenic animals have been produced, our studies are the only experiments examining lactose and milk production in transgenic swine over expressing α-lactalbumin (Bleck et al., 1998; Noble et al., 2000a,b).

Ⅱ. MATERIALS AND METHODS

1. Production of Transgenic Pigs

Duroc, Yorkshire, and Duroc X Yorkshire gilts were injected with PG 600 (Intervet, Millsboro,

DE) at 170 to 210 days of age. Gilts that responded to the injection by exhibiting standing estrus continued in the study. These animals were injected with PMSG (Sigma Chemical Co., St. Louis, MO) 16 days after standing estrus and then injected with hCG 72 h after the PMSG injection. Animals exhibiting standing estrus were artificially inseminated. Embryos were collected 54 h after hCG injection by surgical embryo collection from the oviduct. Embryos were flushed from the oviduct using Beltsville embryo culture medium (Dobrinsky et al., 1996). The embryos were centrifuged at 15,000×g for 5 to 10 min. to visualize the pronuclei and a single pronuclei was microinjected with the DNA construct (Fig. 1). The injected DNA was at a concentration of approximately 4 ng/μL in microinjection buffer (10 mM Tris, 0.1 mM EDTA, pH 7.4). Approximately 20 normal appearing injected embryos were transferred to each recipient animal. Recipient gilts were animals showing standing estrus within a day of the donor animal.

2. Screening of Transgenic Animals

DNA from tail biopsies was extracted (Hogan et al., 1986). Polymerase chain reaction (PCR) was performed using 10μl 10x PCR reaction buffer (500 mM KCl, 100mM Tris-HCl (pH=8.8), 15 mM $MgCl_2$, 1% Triton X-100), 200 mM each dNTP, 1.0 μM each primer (Primers 1 and 2 spanned a portion of the α-lactalbumin promoter and coding sequence; Fig. 1), 1 unit Taq DNA polymerase and 1μg genomic DNA. Volume was adjusted to 100 μl with double distilled filter sterilized water and reaction was overlaid with light mineral oil. Samples were subjected to 30 cycles (94℃ 2min., 50℃ 1.5min., 72℃ 1.5min.). PCR products were separated in an 1% agarose gel and stained with ethidium bromide. DNA from transgenic pigs contains a 480 bp band from PCR corresponding to a portion of the bovine α-lactalbumin 5' flanking region.

3. Milk Collection

Piglets were separated from the sow for 1 hour, two hours after the sow has finished feeding. All sows were fed at 7:00 am each day. This acted to standardize the interval from feeding to milk collection. After the separation the sow was injected intravenously with 1.0 I.U. of oxytocin. The sow was then milked by hand.

4. Expression of α-Lactalbumin in Milk by ELISA

A dilution (1/10,000) of human α-lactalbumin antiserum (Sigma, St. Louis, MO) was coated on 96-well ELISA plates for sandwich-type assays. Plates were rinsed with PBS and then 50μl MOPS-buffered saline is added. α-lactalbumin standards or samples, in 50μl assay buffer, were pipetted into wells. Fifty μl of diluted α-lacta-

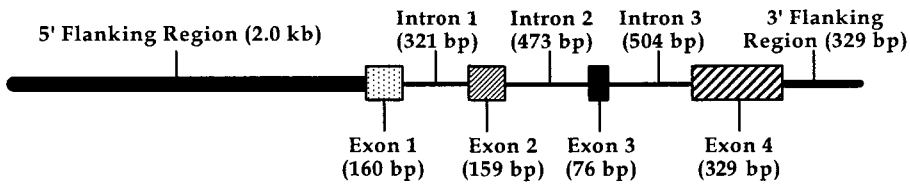

Fig. 1. Structure of the microinjected bovine α-lactalbumin gene construct. The 5' flanking region is approximately 2.0 kb, the coding region (exons and introns) is 2.0 kb and the 3' flanking region is 329 bp.

lbumin-LC -biotin was added into each well. Plates were incubated overnight at 4℃. After the competitive incubation, plates were washed, 100 μl of avidin-peroxidase (1/10,000) was added and plates incubated for two hours at room temperature. Plates were washed and 125 μl substrate buffer (19.7ml 0.05M sodium acetate, pH 4.8, 0.25ml tetramethylbenzidine, 20mg/ml DMSO, 0.08ml 0.5M hydrogen peroxide) was added to each well. After a 20 minute incubation with peroxidase substrate, 50 μl of 0.5M sulfuric acid was added to each well to stop the reaction. Absorbance was then determined at 450~600 nm by an ELISA reader. Sensitivity of the assay was 0.2ng/ml, and the usable range of the assay is between 1 and 100 ng/ml. The intra-assay and inter-assay coefficients of variation for this assay were typically 6~8%. This ELISA specifically recognizes bovine α-lactalbumin and shows no cross reactivity with the porcine protein.

5. Urea PAGE, SDS-PAGE, and Western Blotting

The porcine and bovine milk samples were separated on 14% Urea-PAGE non-reducing gels (Kim and Jimenez-Flores, 1993) or 15% SDS-PAGE reducing gels (Laemmli, 1970). Gels were either stained with Coomassie Brilliant Blue R-250 or transferred onto Immobilon-P membranes (Millipore, Bedford, MA) for western blotting. Western blots were performed using methods of Mao and Bremel (1991). Human α-lactalbumin antiserum (Sigma Chemical Co.) was used to probe the western blots. The human antiserum recognizes both bovine and porcine α-lactalbumin on the western blots.

6. Quantitation of Western Blots

Quantitation was performed by scanning the blots on a Epson ES-1200C color scanner. The resulting images were analyzed using the Collage^{tm} image analysis software (Fotodyne, Hartland, WI). The number and intensity of the pixels in the resulting band were quantitated.

7. Milk Analysis (Lactose, Fat, Protein, Total Solids)

Lactose content of milk was measured by a colorimetric assay based on the procedure of Teles (Teles et al., 1978). Fat content of the milk was analyzed using the choloroform/methanol extraction method (Bligh and Dyer, 1959). Protein concentration is calculated using the BCA protein assay (Pierce, Rockford, IL). Total solids were measured by scalding and drying 0.5g of milk overnight in an oven at 100℃.

8. Weigh-Suckle-Weigh

This method is generally considered as the most precise to estimate milk volume, especially with refinements (Lewis et al., 1978). Milk yield from each sow was measured on days 3, 6, 9 12 and 21 of lactation by weighing the pigs before and after suckling, as described (Lewis et al., 1978). Standardized litters of ten were used. After separation of the litter from the sow for 1 hr, the pigs were weighed as a group and then allowed to suckle until the first piglet stopped suckling. All sows were fed at 7:00 am each day. Piglets were separated from the sow for 1 hour beginning two hours after the sow finished her feeding. This acts to standardize the interval from feeding to milk collection. Following suckling, the pigs were separated, weighed again, and kept separate until the next hourly suckling period. This scheme was repeated for 5 hours. The difference in weights before and after suckling was an estimate of milk production.

9. Piglet Growth

An additional estimate of the functional impo-

rtance of increased milk production and/or composition is the total weight of the litter. Litter sizes were adjusted to ten by withdrawing or adding to the litter to minimize any effects due solely to litter size. Piglet and litter weight was recorded at birth. Piglet and total litter weights were recorded daily until day 21 after birth. Birth weight was subtracted from ending weight to calculate net gain from birth to the particular time point of interest. Sow weights were recorded when the sow entered the farrowing house and at weaning. This was done to measure sow weigh loss during lactation.

Ⅲ. RESULTS

1. Production of Transgenic Pigs Containing Bovine α-Lactalbumin Gene Constructs

We have successfully produced five lines of transgenic pigs containing bovine α-lactalbumin gene constructs. DNA was isolated from ear biopsies for each of the piglets. PCR was perfo-rmed using two separate primer sets specific for the bovine α-lactalbumin 5' flanking region. This transgene has been inherited in a normal Mendelian fashion in F_1 crosses (Bleck et al., 1996, 1998). The first two lines have also been mated to produce homozygous individuals. Tissue collected from 9 transgenic animals showed that expression of the transgene was specific to the mammary gland and not in other tissues.

2. Production of Bovine α-Lactalbumin in the Milk of Transgenic Pigs

Milk samples were collected from one line of transgenic pigs. In the analysis, five hemizygous transgenic pigs were age, breed and breeding season matched with eight control pigs. Milk samples were collected daily (days 0~21) during the lactation. A non-reducing urea PAGE system was used to separate bovine from porcine α-lactalbumin (Bleck et al., 1998). In this system bovine α-lactalbumin produced by the transgenic pigs migrated at two different locations. This altered mobility does not appear to be explained by differences in sows or stage of lactation. The cause of this mobility shift is currently being investigated. Sows hemizygous for the transgene produced an average of 0.68 g of bovine α-lactalbumin per liter of pig milk on day 0 of lactation (Bleck et al., 1998). The production of the bovine protein causes approximately a 50 % increase in the total α-lactalbumin concentration of pig milk throughout lactation (although the increase was dependent on stage of lactation) (Bleck et al., 1998). The concentration of bovine α-lactalbumin in pig milk was highest on day 0 and 5 of lactation and decreased as lactation progressed (Fig. 2). The reduction of bovine α-lactalbumin in a single animal from day 0 to day 5 is seen in Fig. 3. The ratio of bovine to porcine α-lactalbumin also

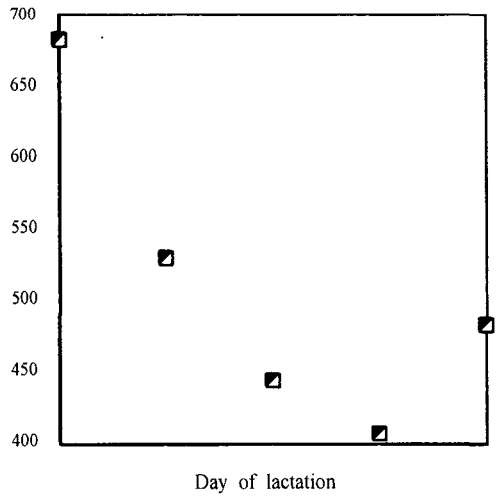

Fig. 2. Bovine α-lactalbumin production in transgenic pig milk during a 20 day lactation (1st parity). Milk samples were collected on days 0, 5, 10, 15 and 20 of lactation. The results are an average of three transgenic sows. (Bleck et al., 1998).

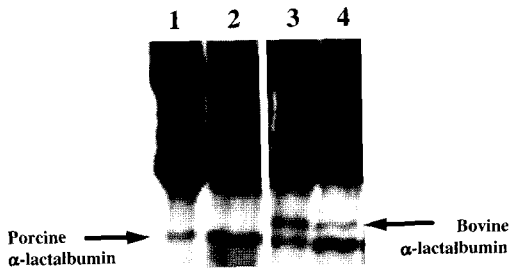

Fig. 3. Transgenic bovine α-lactalbumin is more abundant at the start of lactation than on day 5. Milk from a transgenic pig and her control full sister was separated by non-reducing urea PAGE. Lane 1: Control pig milk day 0 of lactation. Lane 2: Control pig milk day 5 of lactation. Lane 3: Transgenic pig milk day 0 of lactation. Lane 4: Transgenic pig milk day 5 of lactation. (Bleck et al., 1998).

appears to change during this interval.

3. Variation in the Expression of Bovine α-Lactalbumin in Milk Throughout Lactation

To compare the relative concentrations of porcine and bovine α-lactalbumin milk from five first lactation transgenic sows was analyzed. The ratio of bovine α-lactalbumin to porcine α-lactalbumin was 4.3 to 1 on day 0 of lactation, but by day 20 of lactation the ratio was 0.43 to 1 (Bleck et al., 1998) suggesting that the bovine transgene and the endogenous porcine gene are under slightly different control mechanisms. These ratios were calculated by densitometry of the western blot in Fig. 4.

4. 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

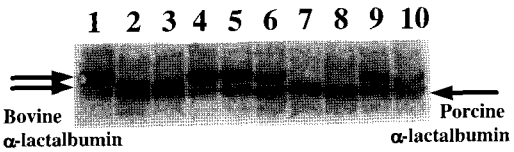

Fig. 4. Western blot of a non-reducing urea PAGE of transgenic pig milk. Milk samples were collected from five first parity transgenic sows. Lanes 1~5 milk samples from day 0 of lactation. Lanes 6~10 milk samples from day 20 of lactation. (Bleck et al., 1998).

transgenic and control sows decreased as lactation advanced, reaching a relatively constant level at day 10 of lactation. This pattern is similar to that observed for the concentration of bovine α-lactalbumin, suggesting that expression of the transgene is regulated in a manner analogous to most porcine milk proteins. There was a trend for the transgenic animals to have a lower protein percentage 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 significantly higher total solids percentage than control sows on days 10 and 20 of lactation ($P<0.01$). However, this difference was not consistent throughout lactation (Bleck et al., 1998).

5. Lactose Concentration of Control and Transgenic Pig Milk

The higher level of total α-lactalbumin present on day 0 of lactation (Bleck et al., 1998) was correlated with higher lactose percentage on day 0 in transgenic sows (3.8 %) as compared to controls (2.6 %) ($P<0.01$) (Fig. 5). There was a trend for higher lactose percentage in transgenic sows also on days 5 and 10 of lactation, but no significant differences were observed. The significant difference on day 0 of lactation was also observed in the

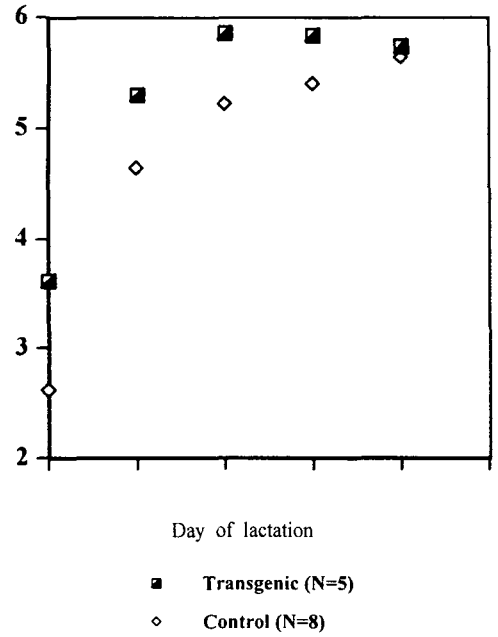

Fig. 5. Average milk lactose percentage from five first parity sows compared to eight control sows over a 20 day lactation (first parity). Lactose percentage was significantly different between transgenic and controls on day 0 of lactation (P<0.01). A trend for higher lactose percentage in the transgenic sows was also observed on days 5 and 10, however the difference was not significant. (Bleck et al., 1998)

second lactation of these pigs. Day 0 lactose percentage was 3.7 % for transgenic sows and 2.6 % for control sows (P<0.01). These data suggest that α-lactalbumin is limiting early in lactation of swine. Furthermore it is possible that higher concentrations of α-lactalbumin at early time points in lactation are responsible for boosting lactation causing maximal milk output at an earlier time (Noble et al., 2000a).

Further assessment of α-lactalbumin, lactose, total solids, and total protein content in milk from offspring (n=20 control and 20 transgenic full sib females) of the founder transgenic line are consistent with results found in Bleck et al. (1998). These data are still preliminary and additional animals are being assessed (Noble et al., 2000a).

6. Milk Production of Transgenic Sows

Preliminary results, using weigh-suckle-weigh analysis, milk production by transgenic (n=17) and control gilts (n=20) were assessed on days 3, 6, 9, and 12 of lactation. On days 3, 6, and 9 of lactation, milk production in transgenic gilts were greater than their controls (P<0.01; Noble et al., 2000b).

The growth rate of piglets nursing transgenic sows was compared to those nursing control sows. At weaning (day 21), piglets suckling transgenic sows weighed ~500 g more than piglets suckling the control sows (Noble et al., 2000b). Some very preliminary data indicate that piglets suckling homozygous transgenic females (n=6) are 1000 g heavier than control piglets (O'Callaghan et al., unpublished).

7. Production and Characterization of Insulin-like Growth Factor-I (IGF-I) Transgenic Swine

Three transgenic boars containing a gene construct that will allow production of higher IGF-I levels in milk have been produced (Donovan et al., 2000). They are currently being mated to produce transgenic sows that will allow us to study the effects of this gene on piglet growth and health.

Preliminary results indicate that colostral IGF-I content of transgenic sows ranged from 0.6 to 1.4 mg/L, compared to <0.1 mg/L in non-transgenic milk (Monaco et al., 2000; Donovan et al., 2000). Further, milk IGF-I was maintained at ~0.5 mg/L until day 20 postpartum, approximately 6-fold higher than milk IGF-I content of non-transgenic sows (Monaco et al., 2000; Donovan et al., 2000). Thus, transgenic swine over expressing human IGF-I in milk at concentrations, which have been

shown to be bioactive within the neonatal piglet intestine, have been generated and will be used for future studies investigating piglet growth and intestinal health.

Ⅳ. DISCUSSION

These results demonstrate that the bovine α-lactalbumin gene can be expressed in the porcine mammary gland and the protein can be subsequently secreted 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 faster than controls (Noble et al., 2000b). 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 lactation performance and agalactia in some animals (Shamay et al., 1991; Wall et al., 1991).

The bovine gene construct used in these experiments induced the production of α-lactalbumin at levels that were approximately 50% of normal endogenous porcine α-lactalbumin. The bovine α-lactalbumin produced by sows appeared to be the same size as endogenous bovine α-lactalbumin, however the mobility of α-lactalbumin was altered on non-reducing urea-PAGE gels with some samples. This different mobility was not explained by glycosylation differences and seems to be related to its conformation, since both forms behave the same under reducing SDS-PAGE conditions. It has previously been shown that bovine α-lactalbumin produced in transgenic mice had the same isoelectric point, calcium binding properties, glycosylation pattern, and N-terminal amino acid sequence as endogenously produced bovine α-lactalbumin (Jeng et al., 1997).

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. Contrary to bovine α-lactalbumin, porcine α-lactalbumin was lowest on d 0 of lactation and became higher later in lactation. These data suggested that in these transgenic pigs the bovine α-lactalbumin gene was being regulated differently than porcine α-lactalbumin and was behaving more like other porcine milk proteins. Previous data in transgenic mice containing a similar bovine gene construct showed that the bovine α-lactalbumin concentration in milk was correlated with the mouse lactation curve; the concentration of bovine α-lactalbumin was the highest when the most milk was being produced (Bleck and Bremel, 1994). This was contrary to what was observed in this line of pigs, since at times of highest milk production, the bovine α-lactalbumin concentration was lower. There are a couple of potential explanations for the difference in transgene regulation between mice and pigs other than the simple interpretation that mice and pigs regulate the bovine construct differently. First, the construct used in the pig experiments was approximately 2.7 kb shorter on the 3' end than the construct used in the mouse experiments. By reducing the size of the construct we may have lost regulatory regions that were present in the 2.7 kb fragment and needed for proper expression. Second, only one line of pigs has been analyzed to date. Insertion site of the gene in this line of pigs may be affecting the expression pattern of the transgene. The examination of more transgenic lines will allow us to determine whether or not the pattern of expression observed in the study is unique to this line of pigs or is common in all lines containing the same gene construct.

Since bovine α-lactalbumin was being produced

at higher levels on day 0 and 5 and porcine α-lactalbumin had not yet reached its maximum concentration, total α-lactalbumin of transgenic sows was dramatically elevated in early lactation milk. In individual pigs, the ratio of bovine to porcine α-lactalbumin was as high as 10 to 1 on day 0 of lactation. Considering that lactose percentage in pig milk does not reach a maximum until later in lactation (Klobasa et al., 1987; Zou et al., 1992), there was the potential that lactose synthase was limiting until lactose production reaches its peak. The boost in early lactation α-lactalbumin production should increase lactose synthesis if α-lactalbumin was limiting and GT was in excess at this stage of lactation. The lactose percentage data supported this hypothesis. On day 0 of lactation, a 46% increase ($P<0.01$) in lactose concentration (2.6 vs. 3.8%) between transgenic and control sows was observed. However, by days 5 and 10 of lactation the difference was no longer significant, even though a trend for higher concentration in the transgenic sows was observed.

The level of milk protein and total solids was not significantly affected by the increased lactose concentration on day 0; however, the average concentrations of both were lower in transgenic sows than in controls, though not significant. If a higher lactose concentration would lead to increased milk production, one may expect that the concentration of protein and total solids would be lower due to added water being drawn into milk by osmosis (Stacey et al., 1995). The total solids values in this study were extremely variable and significant differences were observed between genotypes on days 10 and 20 of lactation. Since protein and lactose were measured individually, the variation in total solids was predominantly due to fat variation. One possible explanation of the variation was the method used to collect milk samples. Samples were collected in between suckling periods of the piglets, but the time from the previous period was not consistent for each sample. A potential explanation is that some samples contained milk that more closely resembled residual milk while other samples resembled milk normally obtained by the piglets during a suckling period.

Overall, these data showed that α-lactalbumin level in the milk of the pig can be increased through the use of transgenic swine, suggesting 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. The increase in lactose content in the colostrum and milk of transgenic sows is most likely a result of α-lactalbumin over expression associated with the presence of the transgene. Because lactose is the major osmole in milk, the increase in lactose content is most likely increasing water content, which would most likely cause a slight dilution in total solid, fat, and total protein contents as seen in this study. Overall, the presence of the transgene has the greatest impact on milk composition early in lactation (Noble et al., 2000a).

Ⅴ. SUMMARY

This study suggests that early lactation lactose production may be increased through the over expression of α-lactalbumin in the mammary gland. Higher lactose levels in early lactation would provide the developing piglet with greater energy intake leading to faster growth. Furthermore, due to the role of lactose as the major osmole in milk, increased lactose levels may be associated with greater milk production at the start of lactation.

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