Acta Scientiae Veterinariae, 2011. 39(Suppl 1): s295 - s300.

ISSN 1679-9216 (Online)

Genetic engineering of livestock to improve human health:The human lysozyme transgenic goat model

Elizabeth A. Maga[1] & James D. Murray[1,2]

ABSTRACT

***Background*:** Transgenic animals have been generated for a variety of purposes including research tools, medical models, bioreactors (dairy animals producing human pharmaceuticals in their milk or in the egg whites of chickens) and for production agriculture (animals with increased growth, decreased environmental pollution, disease resistance), which includes the generation of animals designed to benefit human health. For example, transgenic goats expressing human lysozyme in their milk are being used as a model method to supply milk with antibacterial properties to help fight diarrheal illnesses in children. Lysozyme is a naturally occurring antimicrobial found in human milk at much higher levels than in the milk of dairy goats and cows. Lysozyme serves as part of the natural defense system against infection and also helps establish a healthy gut microbiota in the infant. We hypothesized that the presence of increased levels of lysozyme in the milk of dairy goats could offer several benefits that affect human health, including the promotion of a healthy gut microbiota and associated benefits such as improved growth and resistance to intestinal infections.

***Review*:** Research with this line of transgenic goats over the last 12 years has demonstrated that the presence and expression of the human lysozyme transgene is not detrimental to the animals themselves and that the milk can indeed act in an antimicrobial fashion when consumed by pigs, a model animal for human health, and impact the state of the intestine in a positive manner. Pigs consuming milk from human lysozyme transgenic goats had significantly lower levels of coliforms and *E. coli* in their intestine than did pigs consuming milk from non-transgenic control animals. In addition to bacterial changes, intestinal tissue of pigs consuming milk from lysozyme transgenic goats had a significantly larger surface area with significantly fewer intraepithelial lymphocytes and an elevated level of expression of the anti-inflammatory cytokine TGF-â1 compared to control-fed animals, all indicators of a healthier intestinal tract. Metabolite profile analysis demonstrated significant differences in the levels of 18 metabolites in the serum of pigs fed lysozyme milk with the direction of changes beneficial to the health of the animal. Finally, pigs consuming milk from lysozyme transgenic goats were more resistant to infection when challenged with an enteropathogenic *E. coli*, indicating a protective effect of lysozyme milk. In the semi-arid northeast of Brazil, 89 of 1000 children die before they reach the age of 2 years and 17% of these deaths can be attributed to diarrhea. The use of genetically engineered animals containing increased levels of lysozyme in their milk is a novel and simple approach to fight this problem. Work will be presented outlining the characterization of these animals and the impact of consuming the milk with the goal of one day having the milk available as a preventative or treatment agent for diarrheal illnesses.

***Conclusions*:** Genetic engineering is a viable approach to produce animal food products that can be used to improve human health. Brazil's acceptance of this technology has positioned itself at the forefront to demonstrate to the world a new tool to help fight common diarrhea and its impact on the growth and development of children.

Keywords: Genetic engineering, transgenic, lysozyme, diarrhea, goats.

[1]Department of Animal Science and [2]Department of Population Health and Reproduction, University of California. CORRESPONDENCE: E. A. Maga [eamaga@ucdavis.edu]. One Shields Avenue, Davis, CA 95616, U.S.A.

I. INTRODUCTION

The goal of genetic engineering (GE) of domesticated livestock is the same as breeding and selection: the introduction and propagation of a desired trait. In this approach, the genetic change is introduced in the form of a transgene consisting of the DNA encoding the gene of interest coupled to regulatory elements which are designed to express a specific protein in a tissue- and temporally-specific fashion to give the animal the desired trait. The first demonstration of an altered phenotype in an animal via transgenesis came in 1982 when increased growth was reported in transgenic mice expressing a rat growth hormone (GH) transgene [24]. The first reports of GE livestock soon followed in 1985 [13]. Since that time, the use of GE food animals has been focused on two main areas, namely the development of improved animals for production purposes including growth [1,7,13], decreasing the environmental footprint by reducing phosphorous pollution [12] and engineering disease resistance [28,35] and specialized non-agricultural purposes such as using dairy animals and chickens as bioreactors to produce human pharmaceuticals [8,9,15,26] or pigs to produce compatible organs for human transplant [25,34]. Also included is work designed to improve human health through the GE of animal food products including increased protein levels in milk [2], healthier fat composition of milk and meat [16,27], and the expression of antimicrobials in milk [18,19]. We have generated a line of transgenic dairy goats expressing increased levels of the antimicrobial human lysozyme in their milk with the intent of using the milk as a treatment or preventative agent against debilitating childhood diarrhea. Here, we will review work with our human lysozyme transgenic goat model with respect to the health and well-being of the transgenic line as well as work demonstrating the potential of the milk to improve intestinal health.

II. HUMAN LYSOZYME AND THE PROTECTIVE PROPERTIES OF HUMAN MILK

Lysozyme is a naturally occurring antimicrobial protein found in avian egg whites and the tears, saliva and milk of all mammals [reviewed by 22]. Lysozymes are part of the natural defense mechanism against bacterial infection and assist in digestion of intestinal bacteria. Lysozyme specifically catalyzes the cleavage of the glycosidic linkage between the C-1 of N-acetylmuramic acid and the C-4 of N-acetylglucoseamine that make up the peptidoglycan component of bacterial cell walls. Cleavage of the protective peptidoglycan layer by lysozyme causes leakage of the cell's interior components and results in cell lysis. Lysozyme is more effective against gram positive bacteria but also has been demonstrated to kill gram negative bacteria. Lysozyme is naturally present in human milk at concentrations 1600 times greater than in goat milk [5]. Human milk maintains high levels of lysozyme (400 µg/mL) throughout lactation, as opposed to the milk of dairy animals which has high levels of lysozyme at parturition and involution, corresponding to when the animal is most susceptible to infection, thereby offering protection, with bovine milk averaging only 0.130 µg/mL and goat milk 0.250 µg/ml of lysozyme.

Lysozyme, along with lactoferrin and secretory IgA, are considered to be responsible for the passive immunity of human milk and play an important role for the infant by offering defense against bacterial infection by pathogenic organisms, promoting the development and maturation of the intestinal tract, acting as anti-inflammatory agents, and stimulating a beneficial gut microbiota [reviewed by 11,17]. Breast-fed human infants tend to have a more simple gut microbiota comprised primarily of *Bifidobacteria*, along with *Lactobacilli* and *Staphylococci*, while the fecal flora of formula-fed infants is more complex, with *Coliforms*, *Enterococci*, *Bacteroides*, *Clostridia* and *Streptococci* all being prevalent [29]. One reason for the growth of fewer facultative anaerobes in breast-fed infants is believed to be the antimicrobial factors in human milk such as lysozyme and lactoferrin [23]. A probiotic intestinal microbiota is thought to confer a number of positive benefits including growth, protection against diarrhea and other gastrointestinal illnesses [reviewed by 32] and

indeed, breast-fed infants are less afflicted by acute and chronic diseases, including infections of the gastrointestinal, respiratory, and urinary tract than are formula-fed infants [30].

Diarrhea is one of the leading causes of death of children worldwide. According to the WHO, two million children under the age of five die each year from common diarrheal illnesses. In addition, multiple episodes of acute and persistent diarrhea can leave lasting nutritional and cognitive shortfalls. In the semi-arid Northeast region of Brazil, childhood mortality rates are 3 times higher than the rest of the country (89 deaths/1000 births by the age of 2) with death due to diarrhea accounting for 15-20% of the overall deaths. Breastfeeding is the recommended intervention for prevention of diarrhea in young children. A series of studies documented the reduction of diarrhea episodes and also a fast recovery in breast-fed children. If the amounts of important human antimicrobial proteins could be increased in the milk of common dairy animals such as the goat, there would be a continuous supply of milk that mimics the antimicrobial function of human milk.

One means of providing the beneficial properties of lysozyme to human consumers is to GE dairy animals to produce milk with higher levels of lysozyme throughout lactation. The consumption of increased amounts lysozyme would pose little risk to human consumers of the milk as lysozyme is naturally present in saliva and thus already consumed. By producing the human form of lysozyme, no allergic reactions would be anticipated and lysozymes in general are not known to be related to any known allergens or toxins. Indeed, lysozyme from hen egg whites is currently used with Generally Recognized As Safe (GRAS) status as a preservative in the food industry (sprayed on cheeses and meats, in edible films and in cosmetics) to prevent product spoilage caused by bacteria [22]. Lysozyme is also able to retain activity at pasteurization temperatures [31], survive transit, and be active in the environment of the gastrointestinal tract [10].

If lysozyme were expressed at a higher level in a goat mammary gland throughout the duration of lactation, we hypothesized that several benefits could be considered that affect both animal and human health while posing little risk. Because of its antimicrobial nature, lysozyme in milk could reduce the growth of bacterial contaminants in milk that cause disease in humans (*Listeria*) making for a safer product for consumption and also reduce the growth of bacteria that case the spoilage of milk thereby increasing the shelf-life of milk and thus the availability of the nutrients. The presence of an antimicrobial in the udder could also decrease the incidence and severity of mastitis, thereby improving animal health and welfare. Furthermore, due to its purported role in human breast milk, consumption of lysozyme-rich milk could promote a healthy gut microbiota in individuals consuming the milk, thereby imparting health benefits such as improved growth, reduction or cessation of illness, and resistance to new infections. We therefore propose a strategy to improve human health based on local agriculture, whereby milk from GE dairy goats producing increased levels of human lysozyme can be used to treat and/or prevent diarrhea in children of all ages. The work described below directly addresses these possibilities.

III. THE HUMAN LYSOZYME (HLZ) TRANSGENIC LINE

A line of transgenic dairy goats of Alpine and Toggenburg origins was generated using standard pronuclear microinjection with a bovine α_{s1}-casein-HLZ cDNA transgene [18]. To date we have produced, by natural breeding, a total of 82 (37 female and 45 male) hemizygous transgenic goats through the 5th generation carrying and expressing this transgene. This line of animals transmits the transgene in a Mendelian fashion, stably expresses the transgene at the mRNA and protein level across generations, and the HLZ in milk is biologically active [19]. HLZ protein expression in milk of these HLZ transgenic goats averages 270 ± 84 μg/mL [19]. This represents a 1000 fold increase over the mean level of lysozyme normally present in goat milk and is approximately 68% of that normally found in human milk. The percentage of milk yield that represents total fat and protein was the same range as the means for our non-transgenic control dairy goat herd [19], indicating that expression of the transgene did not disrupt the gross composition of milk. Basic functions such as growth and reproduction of the transgenic animals themselves were not adversely impacted by either the presence or expression of the transgene, or by consumption of the HLZ-containing milk [14].

IV. ANTIMICROBIAL ACTIVITY OF HLZ TRANSGENIC GOAT MILK

Several strains of bacteria important to animal health and food safety were susceptible to HLZ transgenic goat milk *in vitro* [20]. When incubated with various bacterial isolates, milk from HLZ transgenic

animals significantly slowed the growth of *S. aureus, E. coli* and *P. fragi* as demonstrated by an overall lower mean number of colony forming units (CFU)/mL after incubation than milk from non-transgenic controls. The growth of a lactic acid bacteria (*L. lactis*) was not affected by the presence of HLZ in milk. Milk from HLZ transgenic animals was also capable of slowing the growth of bacteria *in vivo*. Milk from HLZ transgenic animals was found to have a different bacterial population corresponding to a longer shelf life [20]. Fewer bacteria grew in milk of transgenic animals and milk survived at room temperature for longer periods than control milk before bacterial growth occurred. The differential growth of bacteria in milk from transgenic animals indicates that HLZ expressed in milk is able to act in an antimicrobial fashion to alter the growth of bacteria *in vivo*.

V. IMPACT OF CONSUMING HLZ TRANSGENIC GOAT MILK

As the efficacy of HLZ in milk was confirmed as described above, the biological action of HLZ milk at the level of the intestine after consumption was evaluated by assessing the growth of coliform bacteria in the small intestine in two animal models, the goat and the pig. Pigs represent a monogastric animal with a digestive tract similar to humans. The use of pigs as a relevant human medical model is well documented [33] as pigs are frequently used in cardiovascular and nutritional research. Due to the antimicrobial properties of HLZ and evidence that natural components of milk result in different intestinal microbiota and overall intestinal development, it is our hypothesis that the consumption of milk containing active HLZ will impact intestinal microbiota, and thus intestinal health, and resistance to intestinal illness. The first part of this hypothesis was shown to be correct as pasteurized milk from HLZ transgenic animals was capable of modulating intestinal bacteria in both ruminant and non-ruminant animal models [21]. In the more human-relevant model, weanling pigs receiving pasteurized milk from HLZ transgenic animals for 16 days had significantly lower numbers of coliforms and *E. coli* in their intestine than did pigs fed milk from non-transgenic control animals [21]. These data indicate that HLZ expressed in the milk of dairy animals can indeed be biologically active in the intestine and modulate gut microbiota, much like human milk.

Further work in pigs confirmed the second part of our hypothesis by demonstrating that consumption of pasteurized milk from HLZ transgenic goats resulted in beneficial changes in gut histology and protection against intestinal infection [3]. Pigs fed HLZ milk had fewer numbers of coliforms and *E. coli* in both the duodeneum and ileum than did pigs fed milk from non-transgenic controls, repeating the findings of our first study. Animals receiving HLZ milk had significantly wider villi in the duodenum indicating a healthier gut with increased absorptive area. The number of intraepithelial lymphyocytes per micron of villi height was significantly decreased in the duodenum of HLZ-fed animals, an additional indicator of increased gastrointestinal tract health. Animals fed HLZ milk for a period of 4 weeks and then challenged with a porcine-specific enteropathogenic *Escherichia coli* (EPEC) had significantly lower levels of coliforms and *E. coli* in their ileum than did those receiving milk from non-transgenic control animals, indicating a protective effect of HLZ milk against EPEC infection. In addition, standard CBC analysis indicated that no allergic response was occurring upon consumption of HLZ milk. Furthermore, there was no significant difference in the expression of key pro-inflammatory cytokines (TNF-α and IL-8) in intestinal tissue of pigs consuming HLZ or control milk, indicating that an inflammatory response is not induced upon consumption of HLZ milk [6].

Serum from non-challenged pigs was also subjected to a metabolite profiling analysis [4]. A total of 234 metabolites were quantified (178 known, 56 unknown) with levels of 18 known metabolites and 4 unknown metabolites being significantly different in pigs reared on HLZ milk compared to pigs that received control milk. These differences could be broken down into effects of bacteria, increased growth, healthier gastrointestinal tract, and modulation of the immune system with the direction of changes indicative of a healthier gut. In addition, consumption of HLZ milk significantly increased the expression of the anti-inflammatory cytokine TGF-â1 in the small intestine [6], again indicative of a healthier gut. Taken together, these data strongly suggest that milk from HLZ transgenic goats can be used to improve human health by fighting the high childhood mortality rates associated with common diarrheal illnesses.

VI. CONCLUSIONS

Data collected over the years on most applications of transgenic animals for agriculture indicate that the implementation of GE animals can indeed have a positive impact on animal productivity and sustainability as well as animal and human health.

The HLZ transgenic goat model has produced convincing data that the adoption of GE livestock has the potential to benefit human health with little risk. Future work with this approach will revolve around translating the use of HLZ milk to treat/prevent diarrhea by developing a pig model of infection and generating lactoferrin transgenic goats. Our hope is to contribute a new solution to an old problem and prevent the suffering and deaths associated with common diarrhea in Brazil and throughout the world.

REFERENCES

1 Bleck G. T., White B. R., Miller D. J & Wheeler M. B. 1998. Production of bovine α-lactalbumin in the milk of transgenic pigs. *Journal of Animal Science.* 76(12): 3072–3078.

2 Brophy B., Smolenski G., Wheeler T., Wells D., L'Huillier P. & Laible G. 2003. Cloned transgenic cattle produce milk with higher levels of β-casein and γ-casein. *Nature Biotechnology.* 21(2): 157–162.

3 Brundige D.R., Maga E.A., Klasing K.C. & Murray J.D. 2008. Lysozyme transgenic goats' milk influences gastrointestinal morphology in young pigs. *Journal of Nutrition.* 138(5): 921-926.

4 Brundige D.R., Maga E.A., Klasing K.C. & Murray J.D. 2010. Consumption of pasteurized human lysozyme transgenic goats' milk alters serum metabolite profile in young pigs. *Transgenic Research.* 19(4): 563-574.

5 Chandan R.C., Parry R.M. & Shahani K.M. 1968. Lysozyme, lipase, and ribonuclease in milk of various species. *Journal of Dairy Science.* 51(4): 606-607.

6 Cooper C.A., Brundige D.R., Reh W.A., Maga E.A. & Murray J.D. 2011. Lysozyme transgenic goats' milk positively impacts intestinal cytokine expression and morphology. *Transgenic Research.* DOI 10.1007/s11248-011-9489-7.

7 Du S. J., Gong Z., Fletcher G., Shears M., King M.J., Idler D.R. & Hew C.L. 1992. Growth enhancement in transgenic Atlantic salmon by the use of an "all fish" chimeric growth hormone gene construct. *Biotechnology.* 10(2): 176–181.

8 Ebert K.M., Selgrath J.P., DiTullio P., Denman J., Smith T.E., Memon M.A., Schindler J.E., Monastersky G.M., Vitale J.A. & Gordon K. 1991. Transgenic production of a variant of human tissue-type plasminogen activator in goat milk: generation of transgenic goats and analysis of expression. *Biotechnology.* 9(9): 835-838.

9 Edmunds T., Van Patten S.M., Pollock J., Hanson E., Bernasconi R., Higgins E., Manavalan P., Ziomek C., Meade H., McPherson J.M. & Cole E.S. 1998. Transgenically produced human antithrombin: structural and functional comparison to human plasma-derived antithrombin. *Blood.* 91(12): 4561-4571.

10 Goldman A.S., Garza C., Johnson C.A., Nichols B.L., & Goldblum R.M. 1982. Immunologic factors in human milk during the first year of lactation. *Journal of Pediatrics.* 100(4): 563-567.

11 Goldman A.S. 2007. The immune system in human milk and the developing infant. *Breastfeed Medicine.* 2(4): 195-204.

12 Golovan S.P., Meidinger R.G., Ajakaiye A., Cottrill M., Wiederkehr M.Z., Barney D.J., Plante C., Pollard J.W., Fan M.Z., Hayes M.A., Laursen J., Hjorth J.P., Hacker R.R., Phillips J.P. & Forsberg C.W. 2001. Pigs expressing salivary phytase produce low-phosphorus manure. *Nature Biotechnology.* 19(10): 741-745.

13 Hammer R.E., Pursel V.G., Rexroad Jr. C.E., Wall R.J., Bolt D.J., Ebert K.M., Palmiter R.D. & Brinster R.L. 1985. Production of transgenic rabbits, sheep and pigs by microinjection. *Nature.* 315(6021): 680-683.

14 Jackson K.A., Berg J.M., Murray J.D. & Maga E.A. 2010. Evaluating the fitness of human lysozyme transgenic dairy goats: Growth and reproductive traits. *Transgenic Research.* 19(6): 977-986.

15 Kuroiwa Y., Kasinathan P., Sathiyaseelan T., Jiao J.A., Matsushita H., Sathiyaseelan J., Wu H., Mellquist J., Hammitt M., Koster J., Kamoda S., Tachibana K., Ishida I. & Robl J.M. 2009. Antigen-specific human polyclonal antibodies from hyperimmunized cattle. *Nature Biotechnology.* 27(2): 173-181.

16 Lai L., Kang J.X., Li R., Wang J., Witt W.T., Yong H.Y., Hao Y., Wax D.M., Murphy C.N., Rieke A., Samuel M., Linville M.L., Korte S.W., Evans R.W., Starzl T.E., Prather R.S. & Dai Y. 2006. Generation of cloned transgenic pigs rich in omega-3 fatty acids. *Nature Biotechnology.* 24(4): 435-436.

17 Lonnerdal B. 2003. Nutritional and physiologic significance of human milk proteins. *American Journal of Clinical Nutrition.* 77(6): 1537S-1543S.

18 Maga E.A., Sargent R.G., Zeng H., Pati S., Zarling D.A., Oppenheim S.M., Collette N.M.B., Moyer A.L., Conrad-Brink J.S., Rowe J.D., BonDurant R.H., Anderson G.B. & Murray J.D. 2003. Increased efficiency of transgenic livestock production. *Transgenic Research.* 12(4): 485-496.

19 Maga E.A., Shoemaker C.F., Rowe J.D., BonDurant R.H., Anderson G.B. & Murray J.D. 2006. Production and processing of milk from transgenic goats expressing human lysozyme in the mammary gland. *Journal of Dairy Science*. 89(2): 518-524.

20 Maga E.A., Cullor J.S., Smith W., Anderson G.B. & Murray J.D. 2006. Human lysozyme expressed in the mammary gland of transgenic dairy goats can inhibit the growth of bacteria that cause mastitis and the cold-spoilage of milk. *Foodborne Pathogens and Disease*. 3(4): 384-392.

21 Maga E. A., Walker R.L., Anderson G.B. & Murray J.D. 2006. Consumption of milk from transgenic goats expressing human lysozyme in the mammary gland results in the modulation of intestinal microflora. *Transgenic Research*. 15(4): 515-519.

22 Masschalck B. & Michiels C.W. 2003. Antimicrobial properties of lysozyme in relation to foodborne vegetative bacteria. *Critical Reviews in Microbiology*. 29(3): 191-214.

23 Mountzouris K.C., McCartney A.L. & Gibson G.R. 2002. Intestinal microflora of human infants and current trends for its nutritional modulation. *British Journal of Nutrition*. 87(5): 405-420.

24 Palmiter, R.D., Brinster R.L., Hammer R.E., Trumbauer M.E., Rosenfeld M.G., Birnberg N.C. & Evans R.M. 1982. Dramatic growth of mice that develop from eggs microinjected with metallothionein-growth hormone fusion genes. *Nature*. 300(5893): 611-615.

25 Phelps C.J., Koike C., Vaught T.D., Boone J., Wells K.D., Chen S.H., Ball S., Specht S.M., Polejaeva I.A., Monahan J.A., Jobst P.M., Sharma S.B., Lamborn A.E., Garst A.S., Moore M., Demetris A.J., Rudert W.A., Bottino R., Bertera S., Trucco M., Starzl T.E., Dai Y. & Ayares D.L. 2003. Production of alpha 1,3-galactosyltransferase-deficient pigs. *Science*. 299(5605): 411-414.

26 Rapp J.C., Harvey A.J., Speksnijder G.L., Hu W. & Ivarie R. 2003. Biologically active human interferon alpha-2b produced in the egg white of transgenic hens. *Transgenic Research*. 12(5): 569-575.

27 Reh W.A., Maga E.A., Collette N.M.B., Moyer A., Conrad-Brink J.S., Taylor S. J., DePeters E. J., Oppenheim S., Rowe J. D., BonDurant R. H., Anderson G. B. & Murray J. D. 2004. Using a stearoyl-CoA desaturase transgene to alter milk fatty acid composition. *Journal Dairy Science*. 87(10)**:** 3510-3514.

28 Richt J. A., Kasinathan P., Hamir A. N., Castilla J., Sathiyaseelan T., Vargas F., Sathiyaseelan J., Wu H., Matsushita H., Koster J., Kato S., Ishida I., Soto C., Robl J. M. & Kuroiwa Y. 2007. Production of cattle lacking prion protein. *Nature Biotechnology*. 25(1): 132-138.

29 Salminen S., Bouley C., Boutron-Ruault M.C., Cummings J.H., Franck A., Gibson G.R., Isolauri E., Moreau M.C., Roberfroid M. & Rowland I.R. 1998. Functional food science and gastrointestinal physiology and function. *British Journal of Nutrition*. 80(1): S147-S171.

30 Schack-Nielsen L. & Michaelsen K.F. 2007. Advances in our understanding of the biology of human milk and its effects on the offspring. *Journal of Nutrition*. 137(2): S503-510.

31 Shahani K.M., Chandan R.C., Kelly P.L. & MacQuiddy E.L. 1962. Determination of lysozyme in milk and factors affecting its concentration and properties. *Proceedings of the 16th International Dairy Congess*. 8(X): 285-293.

32 Solis B., Samartin S., Gomez S., Nova E. de la Rosa B. & Marcos A. 2002. Probiotics as a help in children suffering from malnutrition and diarrhoea. *European Journal of Clinical Nutrition*. 56(3): S57-S59.

33 Swindle M. M. 1992. In: *Swine as Models in Biomedical Research.* Ames, Iowa: Iowa State University Press.

34 Tai H.C., Ezzelarab M., Hara H., Ayares D. & Cooper D.K. 2007. Progress in xenotransplantation following the introduction of gene-knockout technology. *Transplant International*. 20(2): 107-117.

35 Wall R.J., Powell A.M., Paape M.J., Kerr D.E., Bannerman D.D., Pursel V.G., Wells K.D., Talbot N. & Hawk H.W. 2005. Genetically enhanced cows resist intramammary *Staphylococcus aureus* infection. *Nature Biotechnology*. 23(4): 445-451.