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Prospects for Recombinant Protein Production in Dairy Cattle

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유우로부터 재조합단백질 생산에 대한 전망

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요 약

유우의 유전적 개량에 대한 역사적인 계획이 이형접합적 기능획득을 이루어낸 새로운 유전공학 방법과 연계되어 논의되고 있다. 낙농산업에 유동성을 주기 위해 그 개념은 이미 체계가 세워진 지방조성과 단백질 조성 사이의 유전적인 상관관계를 깨뜨리는데 중점을 두고 설명되고 있다. 부가가치 유전학의 개념이 도입되고, 가축 유선의 금전적 경쟁력이 포유동물 세포배양과 세균발효기술과 연관있는 것으로 여겨지고 있다.

Ⅰ. INTRODUCTION

In the United States the margin on milk prices at the farm level have continued to shrink and the dairy industry is being placed under increasing economic stress. Milk production capability of dairy cattle has increased at a substantially faster rate than the niarket for those products and government programs are viewed by some as not having been helpful and are in the process of being substantially restructured. Even with milk prices remaining significantly above world market prices, the economic prospects for milk producers are not good. If the advances in biotechnology and genetic engineering could in some way be harnessed within the udder of cows it could provide a number of alternatives to commodity milk production as well as provide an opportunity to change the commodity itself. The recent release of "Points to Consider" for production of therapeutics for use in human medicine in milk (3) has given guidance to those companies whose activities are focused in this area and should provide an impetus to further developments in the general area. General aspects of transgenesis application is covered by elsewhere in this volume and this article will be confined to aspects of the application of this transgenic technology specifically related to dairy production and the utility of the udder of dairy cows in biotechnology manufacturing. It will also briefly touch on economic issues and the competitiveness of the technology.

Ⅱ. RESULTS AND DISCUSSION

For nearly a century, fat has been the method of economic evaluation of the milk and the focus of genetics has been on increasing the fat productivity of the animals. As animals were bred to be more efficient, dairy producers could increase the income from their animals by adopting appropriate breeding strategies. Consequently, there is a long standing interest in the variability of these production traits and many studies have been conducted in different countries where these traits have been estimated. Dairy cattle genetics has given us animals efficiently making a product whose value is declining. Fat has fallen into disfavor with the consumer of dairy products and therefore the component which is the historical economic index for milk is no longer an asset to the product itself. It is these underlying economic issues that should drive the application of biotechnology in dairy production because it provides the opportunity for diversification in a saturated market.

The genetics of fat and protein in milk of cattle is remarkably consistent. A summary of a number of genetic estimates of these traits is shown in Table 1 and Table 2. The underlying data used to compile these summaries clearly demonstrate the consistency especially considering that the data were collected over several decades, in different countries and on different breeds of cattle. Particularly striking are the numerical values of the phenotypic and genotypic correlations between milk fat and milk protein content and yield shown in Table 1. The magnitude of the correlation suggests an underlying biological principle is common to the volumetric secretion of milk protein and milk fat. It is perhaps not surprising to find this correlation if enzymes involved in fat synthesis are responding to the same physiological signals as milk proteins.

Table 1. Heritabilities and genetic and phenotypic correlations among yields of milk and milk components(2)*

Trait	Milk	Fat	Protein	Lactose
Milk	.27	.82	.87	.96
Fat	.88	.24	.86	.67
Protein	.95	.93	.27	.81
Lactose	.96	.75	.82	.25

*Heritabilities on the diagonal, genetic correlations above the diagonal and phenotypic correlations below the diagonal

Table 2. Heritabilities and genetic and phenotypic correlations for milk fractional composition(2)*

Trait	Fat %	Protein %	Lactose %
Fat %	.47	.55	.22
Protein %	.49	.48	.02
Lactose %	.11	−.56	.28

*Heritabilities on the diagonal, genetic correlations above the diagonal and phenotypic correlations below the diagonal.

This correlation, based in fundamental biological process, is a constraint for the dairy industry to respond to a changing market. Implied in the correlations is that regardless of how component prices paid for milk at the farm level these economic weights are not in themselves apt to substantially change the composition of milk and lead to a composition of milk more in line with market demands. With phenotypic and genotypic correlations of yield traits for fat and protein having magnitudes form 0.8~0.9 these characteristics will be difficult to change significantly. The equivalent correlations for percent composition are of a smaller magnitude indicating that it might be possible to change these more readily that overall yield . In this regard, it should be recognized that lactose yield will drive water movement and thus overall milk yield, fat and protein with it. The magnitude and sign of the relationships between lactose and fat and protein percentages are highly relevant. In any case, the maximal rate of change of these components under normal selection processes is measured in animal generations. The genetic correlation between fat and protein content certainly gives the opportunity for adjusting the ratio of these components by normal breeding, but it will take a number of generations to have a significant impact. The financial problem facing dairy producers is acute, and a breeding program which will take several decades is not a solution.

While milk has traditionally been valued on its fat content, component pricing is beginning to be implemented in the US. In these component pricing schemes, protein is given a considerably higher value than fat. At the time of this writing, the US component price of milk values protein at $1.825/1b, fat at $.79/1b and other solids at $.54/1b. Thus, within the component pricing system, any incremental increases in protein content of milk have considerably more value than increasing fat. As long as fat has any value at all (i.e. unless there is a penalty on fat) there is no disincentive to continue its production and dairy producers are apt to select for both simultaneously. Clearly, financially stressed dairy producers are not apt to see reduction in fat content in milk as a solution to the excess milk fat on the market.

On the other hand, proteins have value as food constituents apart from dairy products and if the protein content of milk could in someway be elevated through molecular genetic methodology, it would appear that through a component pricing scheme the economic system is already in place to pay producers for adopting the technology. Because there has never before been an opportunity to explicitly change the fat content or the fat: protein ratio in milk, there is little recognition that the energy demands of dairy cattle rations are largely for the synthesis of fat. We have made a simple calculation (5) that if it were possible to reduce fat synthesis in the mammary gland, the production of the current level of milk protein could nearly be sustained on a predominantly forage diet. What the overall performance of animals might be if these changes were imposed will only be determined once such animals can be produced.

Production of transgenic animals who in the heterozygous state exhibit a new characteristic is termed "heterozygous gain of function" One way of considering a heterozygous gain of function of genetic capabilities added to animals through biotechnology would be as "value added" genetic capabilities. In practice, value added genetics relies upon using the very best dairy cattle breeding strategies available. By using these tools in combination with the advances in molecular genetics, the added genetics would provide a boost to the underlying genetic capabilities of the founder animals. To the extent that these capabilities could be discretely targeted it would also imply a segregation of the market for milk. It is assumed that there will always be a market for normal milk and that various quantitative trait loci (QTLs) will be found, and selection programs of various types for yield traits will be maintained. In this way, the on-farm decisions and practices wouldn't change all that much other than additional factors might be considered in the selection of bulls for those producers that might decide to enter a new market area. In a component pricing system, there is little doubt that a bull whose daughters produced 0.5% more protein in their milk would be a very popular animal even if their fat test remained the same or were reduced somewhat.

A simple change which is known to lead to substantlal changes in the manufacturing properties of milk it the kappa casein genotype (4). For reasons which are not clear, animals having

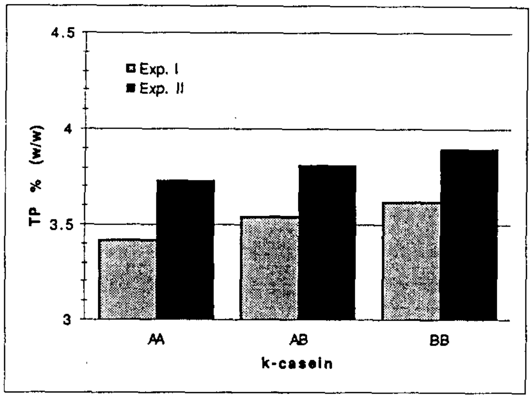

Fig. 1. Milk protein percentage in animals with differing kappa casein genotype(4).

a B allele of the kappa casein molecule produce not only more total protein but also a higher proportion of casein protein than those with an A allele. Moreover, there is a gradation of the phenotype in the order BB>AB>AA. These studies which were done in Holland and included many animals from a number of different sires, there is a clear indication that the B allele of the kappa casein gene is transcribed and translated into protein at a higher rate than the A allele counterpart.

A hindrance to the implementation of a system for production of specialized milks is logistics of milk collection. The tendency in the industry has been to building increasingly large manufacturing plants to take advantage of economies of scale. The production of specialized milks leads to a different situation which may be sufficient to cause changes in the way that milk is procured. For example, as dairies continue to grow in size, economical processing plants might well be established as enlarged milk houses where processing that has traditionally been done in centralized plants would instead be decentralized and carried out at the farm level.

In the future as the technology evolves there will further opportunities for the dairy industry in the production of specialty recombinant proteins. The first of these are apt to be therapeutics of value as human medicines and nutriceuticals or proteins taken orally that have both a nutritional value and a therapeutic value. There are several companies pursuing efforts in this area in the US and Europe. The early target products that these companies are pursuing have a very high value and may have only modest animal numbers required. Because of the small numbers required, it is unlikely that they will have a large impact on dairy production as we know it. However, as the technology matures and the size of markets for products beings to be measured in tons, it is anticipated that herds nomally producing commodity milk will opt to divert their efforts to other products which may have substantially better returns. Dairy farms that opt for this type of operation are apt to be vertically integrated enterprises where animals are owned by the company which produced them along with a production license of some sort. The producer essentially will use them as a production system feeding and milking them for a fair economic return. The points to consider document (3) implies that the system needed for this will be minimally different from a typical Grade "A" dairy operation.

Images of biotechnology conjured up from advertisements tend to contain people dressed in white suits and with a great deal of stainless steel in the background. The production economics associated with these images is startling. A comparison of production costs and several other features of different biotechnological production systems are shown in Table 3. Current methods of recombinant protein production are extremely expensive, typically restricting their use to biomedical applications where the target

Table 3. Economic comparison of mammalian cell culture and bacterial fermentation with expression of recombinant proteins in milk of cows

	CHO	E.Coli	Milk
Product concentration (mg /L)	33.5	460	100
Capital investment	$61M	$389M	$3.3M
Annual expenses	$117M	$242.3M	$0.51M
kg per annum	11.4	11.6	51
Cost per gram	$10,207	$20,912	$10

product's price can be realized. A recent analysis of tissue plasminogen activator production by two different fermentation methods, mammalian cell culture and bacterial culture system is by Datar et al. (1) is telling. This analysis, based on existing production systems, suggests that the production fo these materials will be the range of $10,000 per gram. A large part of the costs of these systems are in the capital investment for the manufacturing plant although the operating costs are also much higher than are typical in agricultural systems. Production costs of this magnitude virtually preclude their use in production systems where patient dosages are any more than a few mg. Certain technologies where patient dosages are estimated in grams are virtually precluded as are production of food materials with a per capita consumption of several pounds per year and a total market in defined in tons. It would seem that burring some enormous advance in fermentation technology, the only way that ton quantities of proteins of desired sequence can be economically produced will be through insertion of the gene coding for it into a normal agricultural commodity. This could be a plant product of some type or, as we propose here, milk. Since it is natural food protein, already in use in its natural state, its use in foods in a recombinant form should be able to gain regulatory approval relatively rapidly. The cost advantage of production in milk is substangial.

Data for CHO cells and E.Coli are taken from Datar et. al (1). Assumptions for milk production are for a typical Wisconcin dairy of 100 cows equipped with new facilities. The capital figures are inflated ~10x that which might be invested in a typical dairy operation which is typically $3000~$4000 per cow.

Several companies have identified the potential to produce customized proteins of high value with medical applications in the milk of domestic livestock. Understandably, their primary focus in towards products required in high volume where the projected lower manufacturing cost provides a competitive edge over existing fermentation technology. At present, this fledgling technology is operating at a very low efficiency of production of transgenic animals If the production of transgenic livestock can be made more efficient, it seems that there anr ample opportunities for adding value to commodity milk and thereby providing new opportunites for the dairy industry. However, unless this efficiency can be improved dramatically it is not likely to see early implementation in an agricultural system.

Ⅲ. SUMMARY

Historical programs for genetic improvement of dairy cattle are discussed in the context of new genetic technologies resulting in a heterozygous gain of function. Concepts are outlined

pointing to the importance of breaking the well established genetic correlation between fat content and protein content of milk to provide flexibility in the dairy industry. The concept of value added genetics is introduced and the economic competitiveness of the mammary glands of livestock are considered in relationship to mammalian cell culture bacterial fermentation technology.

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