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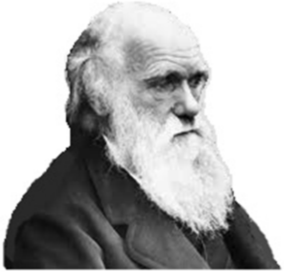

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Base line information on Nutritional profiling of genetically modified forages. A Review

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ABSTRACT

The productivity of animal agriculture in developing countries will need to be substantially increased in order to satisfy increasing consumer demand, to more efficiently utilize scarce resources and to generate income for a growing agricultural population. Biotechnology has the potential to improve the productivity of animals via increasing in growth, carcass quality and reproduction, improved nutritional quality, safety of food, improved health and welfare of animals and reduced waste through more efficient utilization Agricultural biotechnology is one area of application of biotechnology involving applications to agriculture and the application has long been source of innovation in production and processing, extremely impacting the sector. Agricultural biotechnology has been practiced for a long time, as people have sought to improve agriculturally important organisms by selection and breeding. This includes plant breeding to raise and stabilize yields; to improve resistance to pests, diseases and abiotic stresses such as drought and cold; and to enhance the nutritional content of foods. Biotechnology is being used to develop low-cost disease-free planting materials for crops such as cassava, banana and potato and is creating new tools for the diagnosis and treatment of plant and animal diseases and for the measurement and conservation of genetic resources. Modern biotechnology has the potential to provide new opportunities for achieving enhanced livestock productivity in a way that alleviates poverty, improves food security and nutrition and promotes sustainable use of natural resources. The major cause of poor livestock productivity in tropical regions of the world is inadequate nutrition. Low-quality forages are a major component of ruminant diets in the tropics. The lack of quality of ruminant feeds is caused by a high content of lignified crop residues and mature grasses, usually associated with a low content of nitrogen, phosphorus and sulfur. The shortage of feed in most developing countries and the increasing cost of feed ingredients mean that there is a need to improve feed utilization. Animal feeds and feeding practices are being changed by biotechnology to improve animal nutrition and to reduce environmental waste. The ultimate goal of using biotechnology in animal

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nutrition is to improve the plane of nutrition through the use of enzymes to improve the availability of nutrients from feed and to reduce the wastage of the feed.

Keywords: Amino acid, animal nutrition, biotechnology, improved forage

1. INTRODUCTION

Needs of Biotechnology

Naturally occurring animal, plant or microbial strains have few limitations for them to be utilized for desired products due to- purity of the living stock, production of undesired products, secretion of toxic metabolic by-products, inability to withstand harsh biochemical processes/treatments, higher production cost, susceptible to disease and other environmental conditions (Madan, 2005). The existing technology today enables us to engineer plants and animals making them suitable for maximum production. Living organism has a complex cellular structure, metabolic pathways, genetic make-up, and behavior in the synthetic growth media and understanding these processes can help us to modulate specific process/environmental condition or metabolic pathways to achieve the goal of biotechnology (Takada and Otsuka, 2007).

Advancement in different fields of science has paved ways to solve several issues responsible for lower yield of products. Few of the selected science research areas contributing into the development of biotechnology are given in the Figure 1

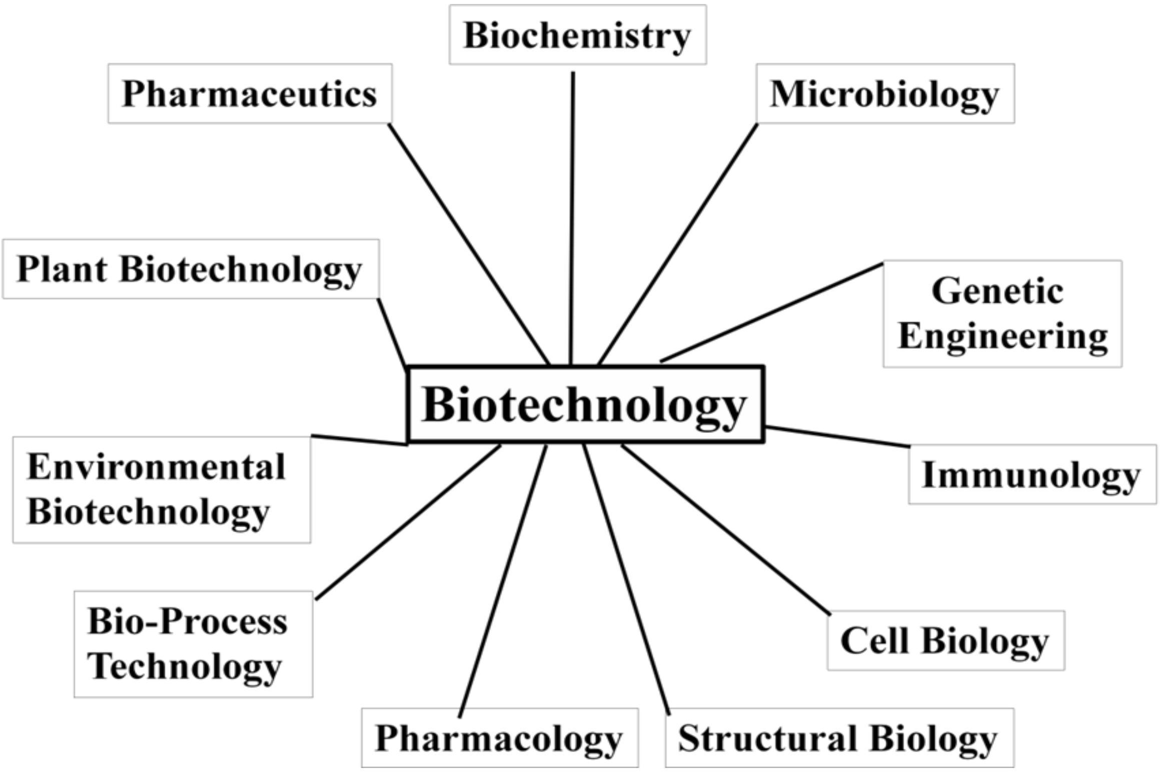

Figure 1. Different Science fields contributing into the advancement of biotechnology

The foundation of biotechnology relies on the research & development activities in different areas of science and interaction of interdisciplinary areas (CERA, 2016). The research in the field of plant biotechnology allowed us to produce plants through micro-propagation but with the evident advancement of genetic engineering, it is now possible to produce plant with predefined characteristics imprinted at genetic level through genetic engineering (Fuller, 2004). The similar relationship may also exist for many other overlapping areas and as a result biotechnological operation output is amplified several folds (Takada and Otsuka, 2007).

Applications of biotechnology

Biotechnology has influenced human life in many ways by inventions to make his life more comfortable (CERA, 2016). Many scientific fields contribute to biotechnology and in return it gives product for their advancement. Few of the biotechnology applications are given in Figure 2. The brief description of application of biotechnology in different field is as follows.

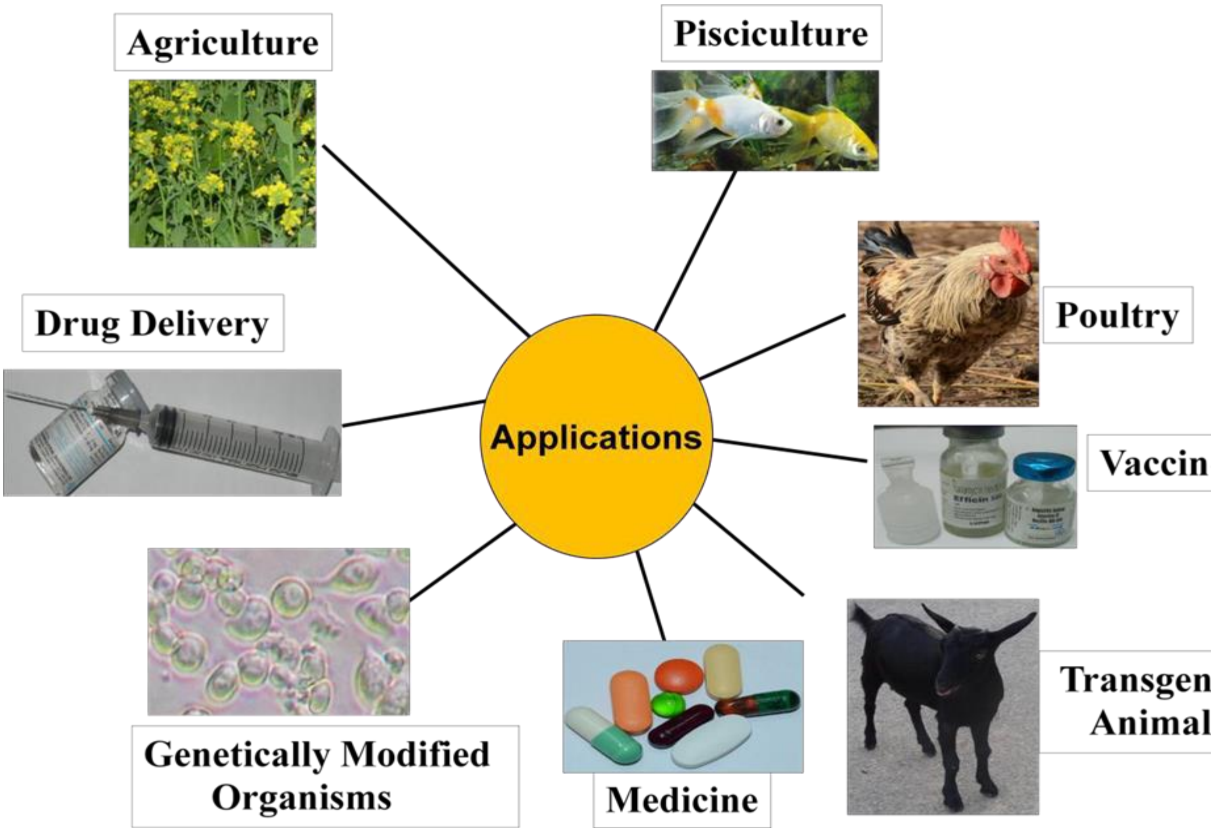

Figure 2. Impact of Biotechnology on different fields & human life.

Animal sciences

One of the early applications of biotechnology in animal science is developing method to separate cheese and other food products from milk by enzyme and microbes (Takada and

Otsuka, 2007). Genetic engineering in conjugation with cell biology and biochemistry has developed multiple products of animal origin. Transgenic animal strains with desired phenotype such as high milk yielding animals, fishes and hens with more fat content (Ravindran *et al*., 2002).

Medicine and Medical Sciences

Biotechnology helped identification of drug like molecules, antibiotics and other medicines. At present a number of antibiotics are being produced by fermentation or in cell based systems (CERA, 2016). Apart from antibiotic, vaccine, diagnostic kits and other immunotherapy are gift of biotechnological advancement (Nyannor *et al*., 2009). Development of artificial limb, arms, heart and medical procedures to perform open-heart operation, dialysis, artificial insemination, test-tube baby and other medical procedures(Takada and Otsuka, 2007).

Plant sciences

Genetic Engineering has allowed us to produce genetically modified plants with diversified properties such as resistance against pest, drought and abiotic stress (Nyannor *et al*., 2009). It has enabled us to produce edible plants with short life-span or ability to grow in different season to increase the number of crops in a year to ultimately increase the food production. Horticulture has used biotechnology tools to produce plants with multiple color, shades, aroma to increase the production of natural colors and scent (CERA, 2016).

Rumen manipulation biotechnology

Ruminants typically features a number of challenges on feeding, because plant matter is difficult to digest, relatively low in fat and protein, and the majority of nutrients are located within strong cell wall fraction (Nyannor *et al*., 2009). As a result, considerable research efforts have focused on methods to modify ruminal fermentation. Rumen biotechnology has the potential to improve the nutritive value of ruminant feedstuffs that are fibrous, low in nitrogen and of limited nutritional value for other animal species. Manipulation of ruminal fermentation involves improving ruminant productivity by maximizing the efficiency of feed utilization performance (Nyannor *et al*., 2009). One approach to achieve nutrient synchrony that has received considerable attention is the manipulation of dietary carbohydrate and protein sources (Takada and Otsuka, 2007). The regulation of fermentation products made by the rumen microbial population would be another target area for use of genetically engineered micro-organisms. The use of genetically modified microorganisms is one area for manipulation of rumen fermentation to change the rumen digestion of specific dietary component.

Protozoa, unlike bacteria, are not vital for the development and survival of the ruminant host, and their elimination (defaunation), although producing a less stable rumen environment, has been found to reduce gaseous carbon and nitrogen losses (Fuller, 2004). It has been established that ruminants can survive with or without these organisms; however, manipulating their population may affect protein metabolism in the rumen. The control of the rumen protozoal population by inhibition compounds would seem attractive because their eukaryotic cell nature would allow them to be susceptible to a number of compounds that would have little or no effect on the prokaryotic bacterial cells. A study indicated, defaunation did not decrease total free amino acid concentrations in ruminal fluid, but it altered the profile of free amino acids (Madan, 2005). An important implication of this study is the possibility of developing a practical way to maintain a reduced number of protozoa in ruminants while at the same time being a source of nutrients (Von *et al*., 2003).

Dry roughage improvement

Fibrous feeds of low digestibility comprise the major proportion of feeds accessible to most ruminants under smallholder situations in developing countries. It is well recognized that some micro-organisms, including cellulose enzymes from anaerobic bacteria and white rot fungi (Pleurotus ostreatus) can degrade lignin in the cell walls (Von *et al*., 2003).

The possibility of biological methods of roughage treatment has a great appeal as an alternative to the use of expensive chemicals and pollution can also be minimized. Several fungal strains have been used for lignocellulosic hydrolysis such as Asprigullus niger, A. terreus, Fusarium moniliforme and Chaetomium celluloyticum (Von *et al*., 2003). However, among many species of fungi white rot fungi have been reported to be suitable for treatment of roughages so far. Found that, the white rot fungi have the capacity to attack lignin polymers, open aromatic rings and release low molecular weight fragments (Fuller, 2004).

Significant results were reported for CP of maize cob treated with fungi species (Pleurotus pulmonarius and Pleurotus sajor-caju). It must be remembered, however, that whatever organism is grown on the roughage must obtain its energy from the roughage itself. In general, the organisms that suit for this purpose must have a number of special properties (Takada and Otsuka, 2007). They must be capable to grow on a wide range of carbon sources, have high growth rates to minimize the size of the fermentation system and have a high efficiency in converting of substrate to biomass with high protein content (Von *et al*., 2003).

Another indirect approach to the enhancement of fibre digestion in ruminants is through modification of silage inoculants. Silage preservation involves the growth of lactobacilli on soluble sugars present in the plants with the production of lactic acid. In silages containing low carbohydrate contents, inclusion of amylase, cellulase or hemicellulase enzymes has been shown to increase lactic acid production by releasing sugars for growth of lactobacilli (Mejia *et al*., 2010). Thus, inoculation of silage bacteria genetically modified to produce such enzymes has been proposed to obtain better ensiling and/or pre-digest the plant material in order to lead to better digestibility in the rumen (Von *et al*., 2003). Recombinant Lactobacillus plantarum, a species used as silage starter, were constructed to express alphaamylase, and cellulase or xylanase genes (Mejia *et al*., 2010). The competitive growth and survival of such modified lactobacilli in silage has been reported by other workers, although the impact on silage digestibility has not been investigated.

2. GREEN FORAGE IMPROVEMENT

Some potential applications of GM crop technology are

- Nutritional enhancement: Higher vitamin content; more healthful fatty acid profiles;
- Stress tolerance: Tolerance to high and low temperatures, salinity, and drought;
- Disease resistance: For example, orange trees resistant to citrus greening disease or American chestnut trees resistant to fungal blight;
- Biofuels: Plants with altered cell wall composition for more efficient conversion to ethanol; Phytoremediation: Plants that extract and concentrate contaminants like heavy metals from polluted sites (Mejia *et al*., 2010).

Genetically engineered forage crops, with a variety of potential benefits for production, the environment and human health, are at present being developed. Genetically engineered forage crops are genetically modified using recombinant DNA technology with the objective of introducing or enhancing a desirable characteristic in the plant or seed (He *et al*., 2009).

These genetically engineered crops are aimed at offering a range of benefits to consumers, as well as developers and producers (Mejia *et al*., 2010). Products to be consumed by humans, derived from animals fed on transgenic forage crops, are not themselves transgenic. Thus food products derived from animals fed on transgenic forage crops offering human health benefits may receive different levels of support from the public than the currently obtainable set of transgenic food crops (He *et al*., 2009).

High lignin content reduces the efficiency of feed utilization and thereby reduces animal growth. Conventionally bred forage varieties with reduced lignin are available, but they tend to have weaker stems and poor standability in the field (Von *et al*., 2003). Researchers have developed engineered alfalfa with 20 percent less lignin and 10 percent more cellulose, a combination that makes it more digestible (Nyannor and Adeola, 2008). The ability to modify specific components of fiber biosynthesis may allow scientists to develop reduced-lignin forage that is more digestible and still has the stem strength needed for good field performance. Protein content and feeding quality are being targeted for improvement in biotech crops (Von *et al*., 2003).

It is known that forage legumes are comparatively low in sulfur-containing amino acids and their availability to ruminants is further adversely affected during rumen digestion (Humphrey *et al*., 2002). This leads to the reduction of the optimum for animal growth level of essential amino acids. Plant genetic modification with genes encoding for a sulfur amino acid-rich proteins, resistant to rapid rumen degradation can compensate this deficiency (He *et al*., 2009). Agronomic researchers around the globe are currently using recombinant DNA technology to create new and altered species of plants (Mejia *et al*., 2010).

High-oil corn reduces the amount of feed required for a livestock diet, and this in turn reduces the volume of manure. Furthermore, conventional high-oil crops often have lower yield or protein content than their lower oil counterparts, whereas traits introduced via biotechnology can modify oil accumulation only at specific growth stages and in targeted tissues to minimize such deleterious effects. Biotech modification of the oil composition of feeds, such as raising the level of oleic acid, may also improve the quality of the resulting animal products for processing and human nutrition (Nyannor and Adeola, 2008).

Nutritional quality of genetically modified feed crops

Livestock and poultry demand for feeds is expected to grow in the next 50 years, as food requirements increase parallel with the doubling of human population (Nyannor and Adeola, 2008). Sufficient, nutritious and environment-friendly feed crops are breeding targets to indirectly provide food for an increasing population (Mejia *et al*., 2010).

Genetic modification especially on the purposeful changing of substances in a particular pathway using recombinant DNA techniques, termed as metabolic engineering, is being conducted to generate new varieties with high yielding and nutrition- enhanced traits (Humphrey *et al*., 2002). Nutrition enhancement in crops targets manipulation of levels of proteins and amino acids, fats and oils, vitamins and minerals, carbohydrates and fiber quality, as well as decreasing the levels of undesirable components in major feed crops.

Feed Crops with Improved Proteins and Amino Acids

For animal requirement, most grains do not provide a balanced source of protein due to deficiencies in essential amino acids: lysine, methionine, and tryptophan (Humphrey *et al*., 2002). Biotechnology has been successfully utilized in the development of crops with increased level of limiting amino acids, thus providing alternative to the direct addition of supplemental amino acids in animal diets, as well as reducing N excretion into the environment (Humphrey *et al*., 2002).

GM maize with increased lysine (LY038) was developed by inserting a *cordapA* gene from a common soil bacteria *Corynobacterium glutamicum* (Nyannor and Adeola, 2008). Enhanced production and accumulation of free lysine (Lys) in the GM corn kernel made body weight gain, feed conversion and carcass yields of experimental poultry and swine comparable with animals fed with Lys supplemented diets, and higher than those fed with conventional maize diets (CERA, 2016).

Rats fed with another Lys- enriched maize with the gene sourced from potato, was also found to be safe as conventional maize (Nyannor and Adeola, 2008). Similar result was found in poultry fed with rice expressing transgene *OASAID* with elevated free tryptophan (Trp) in the seeds (Mejia *et al*., 2010).

Body weight and feed efficiency of chicken fed with 55% GM high Trp rice was similar with those fed with conventional rice supplemented with crystalline Trp, but higher in the group fed with un- supplemented control diet (Cheng-Chih *et al*., 2008). Protein-enriched soybean event M703 was found to contain more digestible amino acids lysine, methionine, threonine, and valine, and had a higher level of metabolizable energy than conventional soybean meal in an experiment with cockerels (He *et al*., 2009).

Narrow-leafed lupin (*Lupinus angustifolius*) that express methionine-rich sunflower albumin has a two-fold increase in methionine content. When fed to broilers, the supplemental methionine required in diets containing 25% lupin meal can be reduced by 0.6 g/kg if GM high-methionine lupins are used instead (Takada and Otsuka, 2007).

Feed Crops with Biologically Active Substances

Barley with its inherent high ß-glucan content has not been used as a feed component. However, with the expression of a thermo-tolerant *Bacillus* ß-glucanase that acts on these glucans, GM barley could be a possible alternative or addition to feeds especially in areas where maize cannot be grown for climatic reasons (Humphrey *et al*., 2002). Barley is a more stress-resilient crop than corn. Feeding studies conducted in poultry showed that a barley-based diet with a small addition of GM grain expressing ß-glucanase can provide an alternative to a maize-based diet for broilers based on body weight gain (Ravindran *et al*., 2002).

Human lactoferrin (LF) and lysozyme (LZ) genes were introduced in rice grain for antibacterial and immune-stimulating properties. Antibiotics are used routinely in poultry farms to improve the intestinal microflora as well as to prevent and treat disease. Chickens fed with portions of GM LF or LZ rice as a substitute for antibiotics in poultry diets showed that the effect in the intestine was comparable with those fed with antibiotics (Mejia *et al*., 2010).

In rats and pigs, another GM rice line expressing the human lactoferrin gene was evaluated and results of digestibility experiments showed that the nutritional quality of LF rice is superior to that of conventional rice (Von *et al*., 2003).

Feed Crops with Improved Phosphorus Availability

The element phosphorus (P) is stored in plants as phytate salt. When consumed by monogastric animals such as horse, pig, poultry, cat, dog, among others, it is poorly soluble and utilized in the gastrointestinal tract, when accompanied with high dietary calcium concentration and absence of endogenous phytase (enzyme hydrolyzing phytate bonds that releases elemental P) activity (Von *et al*., 2003). Hence, the undigested phosphates excreted by these animals when accumulated in soil and water leads to phosphorous pollution and organic matter accumulation, eventually reducing oxygen availability in the water (Humphrey *et al*., 2002). In addition, phytic acid (the reactive form of phytate salt) forms insoluble salts with zinc and other cations reducing bioavailability of trace minerals in these animals.

Thus, development of GM crops with phytase enzyme is an important solution to this problem. GM corn expressing the *Escherichia coli*-derived phytase gene when studied with broiler chicks showed that the use of an increasing dietary level of transgenic maize linearly increased dry matter P, calcium (Ca) and nitrogen (N) retention. It shows that the GM corn is as efficacious as the commercial, microbial phytase in P- and Ca- deficient broiler diets and would thus minimize the need for supplemental dietary P (Hu *et al*., 2010). Additional studies showed improved digestive tract physiology, elevated phytase activity, and decrease phytic acid P content (Sebastian *et al*., 1998). *E. coli* phytase gene introduced into rice showed similar results of safety and nutritional availability in experiments on rats (Nyannor and Adeola, 2008).

Genetically modified soybean that express *Aspergillus niger* phytase transgene was tested in broiler chicks in comparison with phytase-supplemented commercial feed (Nyannor *et al*., 2009). On the basis of performance, P retention and excretion, the authors indicated that phytase from GM soybeans gave a positive effect, similar to the one provided by commercial phytase supplement. Tobacco and wheat containing the same gene showed similar beneficial influence on availability in broiler chicks (Cheng-Chih *et al*., 2008). Use of GM canola with phytase gene from *Aspergillus fucuum* in broiler chicks and weaning pigs also showed that bone ash, P and Ca retention were comparable with that of feeds containing commercial phytase supplement (Denbow *et al*., 1998).

Developing Feed Crops with Improved Fatty Acids

Most of the GM crops modified to improve fatty acid content have been used for direct food or for food industry use such as the oleic acid **soybean DP305423**, which has a better oxidative ability for improved food frying performance (Cheng-Chih *et al*., 2008). Safety and nutritional value of the processed meal, hulls and oils from the GM soybean plant determined from experiments in birds showed that it is nutritionally equivalent to non-modified control as shown in body weight, hen-day egg production, egg mass, feed intake as well as egg production and quality (Edwards *et al*., 2000).

Feed Crops with Reduced Toxins and Anti-nutritive Factors

Non-ruminants are adversely affected by anti-nutritive factors in plant tissues including protease inhibitors, tannins, phytohaemogglutinins and cyanogens in legumes, and glucosinolates, tannins and sanapine in oilseed and other compounds in feeds belonging to the Brassica group (Cheng-Chih *et al*., 2008). A combination of genetic engineering and conventional plant breeding should lead to substantial reduction or removal of the major anti nutritive factors in plant species of importance to animal feeds.

Soybeans contain raffinose and stachyose, the antinutritive oligosacharides that cause osmotic problems in laboratory animals (Zhang *et al*., 2000). Genetically modified soybeans that contain these low oligosaccharides were developed (Edwards *et al*., 2000). In an experiment with three conventional soybean meals and five low-oligosaccharide GM soybean meals fed to broilers, the mean raffinose, stachyose, and galactinol levels in the GM soybean was significantly much lower than the conventional soybean meal, and the crude protein and sucrose contents were slightly higher. Additional data showed that true metabolizable energy was also higher in the GM soybean (Brinch-Pedersen *et al*., 2006).

In another experiment using 28 to 32 day-old chicken, the apparent metabolizable energy of the GM soybean is higher compared to the conventional soybean (Denbow *et al*., 1998). A succeeding experiment on broiler chicks fed with 43% GM or non-GM soybean as the sole source of protein showed that the standardized digestibility coefficients of amino acids methionine, lysine, threonine, valine and isoleucine were significantly higher in the GM –fed soybean. These results show the potential nutritional improvements in soybean cultivars that are genetically modified for low oligosaccharide levels (Zhang *et al*., 2000).

Cottonseed meal, a by-product of the cotton industry, has been a component of cattle feeds because of its protein, fiber, and oil content that improves cattle growth and breeding ability. However, cotton seed contains a yellow phenolic pigment gossypol, which at high concentration in the diet, result to panting and reduced livestock performance (Denbow *et al*., 1998). A pioneering work to reduce the gossypol in the cottonseed was conducted through genetic modification that interferes with the expression and activity of δ-cadinene synthase, the enzyme involved in gossypol production. The gossypol content in the foliage and floral parts of GM cotton were not affected maintaining the crop's ability to resist insect pests. This work allows the use of cottonseed to extract edible oil also for human consumption (Parsons *et al*., 2000).

Gaps in Nutrition Enhancement of GM Feed Crops

The preceding overview of nutritionally enhanced feed crops developed through genetic modification provided information on crops and traits that are under field trial or are already in the early commercialization stages (Edwards *et al*., 2000). Nutritionally-enhanced genetically modified feeds have consistently shown efficacy in providing safe and available nutrients to poultry and livestock in various studies. Sufficient and cheap feed stocks are expected to come as more countries are adopting biotech crops. Research on increasing other nutrients in feed crops such as vitamins, minerals, and fats, reducing anti nutrition factors in plant-based feeds, efficient anaerobic fermentation of silage through genetically modified microorganisms will surely contribute to this enterprise (Edwards *et al*., 2000).

Constraints in application of biotechnology in developing countries

Developing countries are faced with the challenge to rapidly increase agricultural productivity to help feed their growing populations without depleting the natural resource base. Biotechnology is regarded as a means to meet both objectives through addressing the production constraints of small-scale or resource-poor farmers who contribute more than 70% of the food produced in developing countries (Edwards *et al*., 2000). Among agricultural and allied fields, animal production and health have probably benefitted the most from biotechnology. Animal production in most developing countries could be increased many fold by finding ways and means of applying already established concepts. The constraints and limitation of biotechnology in animal production in developing countries are due to the facts that livestock production in these areas are practiced under extensive system requiring to change the production system first before biotechnological interventions are made. In addition, the majority (> 80 %) of biotechnological research activities in biotechnology are conducted by large private companies for commercial exploitation to meet the requirements of developed markets and large-scale commercial producers. They are thus unlikely to be very suitable for the conditions of small-scale farmers in tropical regions and this may lead to increasing inequality of income and wealth within countries.

3. CONCLUSION AND RECOMMENDATION

Biotechnology is a support for various fields of agricultural production and processing and offers a range of tools to advance our understanding, management and use of crop and livestock resources for different social and economic benefits of man. Up to now, biotechnology in animal production in developing countries has been applied only in a few areas such as conservation, animal improvement and healthcare in developing countries. Fibrous feeds, including crop residues, of low digestibility constitute the major proportion of feeds available to most ruminants under smallholder situations in developing countries. Hence, improving these kinds of feeds pre- ingestion will help in increasing nutrient availability and reduce waste of resources. Among biotechnological options in roughage feed improvement for developing countries are: biological treatment of roughage which are abundant, use of genetically modified forage crops with enormous merits and possibly modifying the rumen microbial system to make better use of the available feeds. The use of biotechnology to improve post-ingestion quality of fibrous forages is on the verge of delivering practical benefits to ruminant production system in the tropics. In general, adopting biotechnology has benefited by in animal improvement and economic returns to the livestock entrepreneurs and small producers.

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