

# Chapter 2

## Biotechnology and Animal Nutrition

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**Abstract** To fulfill increased world food demand and to overcome the consequences related to natural and industrial changes, scientists have been trying to select and improve both feedstuff and livestock, genetically. Biotechnology, also involving chemical and physical techniques, is applied to nutrition to increase the abundance (availability) of feed and to improve the digestibility of nutrients in those feeds. Additionally, animal nutrition studies are conducted to determine the safety of human food and modeling of some human diseases. Manipulating animal ability to absorb and utilize more nutrients starts from the plant breeders and continues until where those nutrients are utilized in the body. Microorganisms that have symbiotic life with livestock organism are transgenically manipulated to improve nutrition. Adding new genes to feedstuff gives nutritionists more applicational tools for improving nutrition and animal health through feeds. In contrast, application of recombinant DNA technology to farm animals needs more effort and may result in uncontrollable consequences.

### 2.1 Introduction

The success of genetic modification of livestock and their inner and outer environment depends on how scientists quantify desirable traits, select the marker genes for those traits, and redesign them in superior individuals or populations with a cheaper and more effective way than conventional systems. Despite the genetically engineered application benefits such as reducing the cost of production, scarce material and energy usage, and easing the processes by cutting the steps, etc., the public view

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toward biotechnology is always of concern and convincing them of safety may need special attention and efforts as much as efforts on DNA research. Although the current business in biotechnologically invented and sold products is around a couple of hundred billion dollars ranging from biopharmaceutical substances to enzymes, aminoacids, growth promotants, etc. (Demain 2007). In the future, using single cell protein as the only source of protein and amino acids for animals, or transferring the genes that express high amount of certain amino acids, other nutrients, or any other desired traits from various plant or forage sources to one single forage such as alfalfa (Cunnigham 1999) may solve the problem of nutrient deficiencies in livestock.

## **2.2 Recombinant Technology for Digestive System**

### ***2.2.1 Modification of Ruminant Digestive System***

Rumino-reticulum is the main digesting environment in ruminating animals and both microbial and animal's own enzymes and chemicals work together to digest feeds and synthesize new nutrients and other substances. One of the nutrients that ruminal microorganisms digest is cellulose and its bound complexes with other substances. However, microbes and, therefore, rumen environment have limited capacity to digest cellulose substances compared to other polysaccharides such as starch or proteins. Therefore, manipulating ruminal microorganisms and the relative population of certain species might help to increase the digestibility of high woody plants or low quality feeds. In fact, ruminal fermentation is a complex system and manipulation may not always be successful although other variables of the ecosystem are closely controlled. Presumably, manipulation of the rumen system can be achieved by feed manipulation, host animal manipulation, and microbial manipulation (Nagaraja 2012). So many attempts in biotechnology have been made to manipulate the rumen environment. Since microorganisms utilize the major nutrients of plants, such as cell wall polysaccharides, their efficiency is considered manipulable. Basically, four groups of microorganisms occupy the rumen, including bacteria, methanogens, protozoa, and fungi (Nagaraja 2012). Giving one or a couple of them a chance to grow better, or in other words, decreasing the number of one or couple of them was the initial thoughts of manipulation. Lactate utilizing bacteria inoculation in rumen system had a best fit for animals prone to acidosis and such products called probiotics are produced after microbial fermentation commercially in fermenters (DiLorenzo 2011).

Rumen's ability to utilize ingested materials depends highly on how well the fermentation is constructed and how efficiently the products are removed. Some ways of manipulating rumen environment includes reduction of lactic acidosis and methanogenesis, enhancement of acetic, conjugated linoleic, or propionic acid production, manipulation of protein and fat degradation, improvement of microbial protein synthesis, and elimination of undesirable microorganisms such as protozoa and end products (Scheire and Tamminga 1996). Currently, more than 200 species of

bacteria are isolated from the rumen (McAllister et al. 2006). In spite of this diversity, only 20 of them dominate the population being  $10^{15}$  cells/g of digestive fluid in rumen. The majority of rumen bacteria are obligate anaerobes, but the number of facultative anaerobes can reach up to  $10^7$  cells/g of rumen contents (Yokoyama and Johnson 1993). Initially, it was thought was to reduce some species by adding ionophores to diet and disrupt bacteria cell membrane and suppress the gram-positive bacteria population (Cunnigham 1999). In this way, volatile fatty acid production was shifted and efficiency increased. The other method of manipulating the ruminal environment was to add live microorganisms to the rumen, especially using aspergillus and saccharomyces, which resulted in a benefit for production (Gaggia et al. 2010). Rumen protozoa being larger than bacteria occupy more volume than bacteria with less numbers per gram of fluid ( $10^3$ – $10^6$  cells/g). When it was discovered that ruminants can survive without protozoa (Lindsay and Hogan 1972), protozoal predation of bacteria by protozoa attachment to the fiber particles from outer layers (McAllister et al. 2006) and their symbiotic living with methanogens and, therefore, decreasing ruminal protein metabolism (Finlay et al. 1994), attempts were made to decrease or get rid of ruminal protozoa (Hristov et al. 2003).

Like protozoa, yeast cells involved in plant cell wall digestion and may occupy 8 % of the rumen biomass. Methanogens attracted attention previously only for the loss of energy from the ruminant; currently, because of global warming attempts are toward reducing methane emission by culturing and trying to decrease their growth. Additionally, mycoplasmas and bacteriophages are natural residents of the rumen and may share their functions with other living organisms (McAllister et al. 2006).

Identification and determination of the function of rumen microbes is difficult being in semi-purified culture systems. Previous methods of identification and enumeration of rumen microorganisms were dependent on microscopy, substrate utilization, and defining certain parts of the microbial cells which were biased, time-consuming, needed large amount of colonies, and inaccurate. Recently, real-time PCR methods to sequence 16S rRNA gene for bacteria and 18S rRNA for protozoa have been used to determine the rumen inhabitants in their natural ecosystem (McAllister et al. 2006; Nagaraja 2012).

Based on the fact that most gram-positive microorganisms are lactate producers, ionophore antibiotics are used to change their membrane ion exchange barrier with their highly lipophylic activity. Since gram-negative bacteria have some large membrane molecules to resist ionophore actions, propionate to acetate ratio increases, and propionate used as energy source improves the performance. Additionally, ionophore antibiotics indirectly reduce ammonia accumulation and both methane emission and therefore protein efficiency are improved and energy loss is restricted (DiLorenzo 2011). However, public concern toward feed additive antibiotics claiming that they transfer the antibiotic resistance gene to human microbiota resulted in banning the use of those ionophores in animal feeds (Gaggia et al. 2010).

In rumen there are several types of microorganisms actively involved in the digestion of fibrous, starchy, protein parts of the feeds and antinutritive substances that bind the minerals and other nutrients by their exogenous enzymes. The attempt to improve this enzyme activity has focused on three areas: first, the relative

increase in the population of certain bacteria depending on the ingested materials, second determining the genes responsible for synthesizing those enzymes and manipulating them, and third, the addition of exogenous enzymes before the material is ingested. As mentioned earlier, manipulating the feed and animal has attracted less attention than manipulating the ruminal microorganisms and several ways have been attempted other than subtherapeutic antibiotics, including enzymes, direct fed microbials (DFM), or probiotics, prebiotics, and some plant extracts (Beauchemin et al. 2006; Nagaraja 2012).

Whenever a biotechnological agent was discovered and started to be used for increasing animal performance, public concern has been raised about the issue of animal health, safety and welfare, and product safety as in the case of bovine somatotropin (bST).

Direct-fed microbials, like ionophores, came into use for improving health (i.e., reducing sub-acute ruminal acidosis (SARA)) and productivity. *Saccharomyces cerevisiae* was the first yeast implemented in the ruminant feed industry because of its enhancing effect on milk production and live weight gain (Beauchemin et al. 2006). Fibrolytic enzyme addition to diet to manipulate rumen environment has been the one most extensively studied in recent years (Kahi and Rewe 2008; Nagaraja 2012).

While the poultry industry is well acquainted with exogenous enzymes, especially for antinutritive factors or those not produced by the birds' own metabolism, the ruminant industry is still progressing slowly on enzyme addition to diets. Ruminant diets contain more fiber than other animals and generally 40–70 % of the diet constitutes plant cell wall materials. Considering silages are the largest proportion of ruminant diets, even in the highly digestible ration condition it does not have total tract neutral detergent fiber (NDF) digestibility higher than 65 % (Van Soest 1994). It was also proved that low pH rumen environment decreases NDF digestibility (Basalan 2000). Therefore, scientists put their effort to improve the fiber digestibility of fiber by adding cellulose and hemicellulase, either alone or combined with DFM (Beauchemin et al. 2006). Addition of acarbose (inhibitor of alfa-amylase and glucosidase to decrease rate of starch digestion and therefore ruminal acidosis) and amylase (to increase rate of starch digestion) (DiLorenzo 2011) may both be promising applications in certain feeding conditions. Enzymes used in fiber digestion are series of endo and exo-enzymes (Beauchemin et al. 2006) usually originated from either fungi (*Trichoderma*, *Aspergillus*, etc.) or bacteria (*Bacillus* sp.) (Ghorai et al. 2009; Nagaraja 2012).

Effects of enzymes on ruminant performance have been studied extensively and it was found that some of the enzymes may have promising effects; however, most their mode of action is not well known yet (Beauchemin et al. 2006). For example, some fiber digesting enzymes improved the major performance parameters (dry matter intake, average daily gain, and feed efficiency) in beef cattle, but did not improve DM intake or milk production in dairy cattle. Similarly, one enzyme may improve fiber digestion in alfalfa but not in corn silage probably due to the nature of the fiber (cellulose versus hemicellulose or lignin) (Colombatto et al. 2003). Enzymes are proteins and their stability in various acidic and temperature may be questionable and may affect their activity. Therefore, contradictory results in

production trials may be attributed to the supplier or enzyme selectivity to work on rumen environment (Beauchemin et al. 2006; Bhat 2000). Beef cattle diets do not have large amounts of fiber compared to dairy cattle diets and, therefore, fiber digestibility can be undermined (Basalan 2000). However, some studies showed positive effects on increasing ADF and/or ADF digestibility and growth performance of beef calves, especially in high roughage feeding (Beauchemin et al. 2006). Gencoglu et al. (2010) found that total tract organic matter (OM) and NDF digestibility was improved by addition of exogenous amylolytic enzymes in dairy cattle. Earlier dairy cattle studies showed some improvement in milk production but not in milk constituents in different stages of lactation periods; later studies did not see any significant difference in milk production by the addition of fibrolytic enzymes (Vicini et al. 2003). Proteases and lipases can be of major interest because of the protein starch relationship in rumen (Gohari et al. 2009).

DFM can be defined as live culture of bacteria helpful to host animal when introduced in the gastrointestinal tract for improving performance (Kmet et al. 1993; Saad et al. 2012). Many single or a cocktail of microbe strains have been available for the feed industry market over the years, however, their live activity and selectivity is always questionable (Canganella et al. 1996). Most studies of probiotics focus on reducing ruminal acidosis or improving the digestion by not letting the pH to stay long under a certain level in ruminants (Beauchemin et al. 2006). *Lactobacillus* sp, *Bifidobacterium* sp, *Propionobacterium* sp, *Enterococcus faecium*, *Lactococcus lactis*, *Saccharomyces cerevisiae*, *Saccharomyces boulardii*, *Escherichia coli* strain nissle, etc., are more commonly used microorganisms as probiotics (Saad et al. 2012). While probiotics of fungal origin work in adult ruminants well, probiotics from bacterial origin are likely to improve health and production in young and growing calves (Nagaraja 2012). Using lactic acid producing bacteria for elevation of ruminal pH is somewhat contradictory to common beliefs, however, their effects are generally explained as the promotion of lactic acid utilizers. *Selenomonas ruminantium* and *Megasphaera elsdeni* are major lactate utilizing bacteria and their numbers in population can be increased by *Aspergillus oryzae* or *Saccharomyces cerevisiae* by probably producing nutrients as growth promoters for those bacteria (Nagaraja 2012).

Probiotics act in the lower or upper intestinal tract in several mechanisms to improve productivity; including competition with pathogens to exclude them from luminal cell adhesion or to limit their nutrients in the environment, stimulation of immunity, production of antimicrobial substances for antimicrobial activity, increasing the barrier integrity, and regulation of cytokine production to reduce inflammation (Saad et al. 2012). Besides defining the microorganisms in probiotics, expected characteristics such as survival, toxicity, etc., and safety criteria should be presented (Gaggia et al. 2010). DFM application in some phases of livestock production integrations is more visible than in other phases. For instance, since calves were born without ruminal population established, pre-ruminal calves are more prone to exogenous pathogen microorganisms and therefore probiotics administration to them was found to be useful. Additionally, receiving cattle passes through very stressful phases such as dehorning, castration, vaccination, transport, implant application, etc., and therefore, DFM supplementation of animals in that

phase proved to be useful. Furthermore, dairy cattle in transition period (3–4 weeks prior to parturition and 30–70 days postpartum) are susceptible to many metabolic and birth-related diseases and their consequences may require the use of DFM or yeast (Baeuchemin et al. 2006).

### ***2.2.2 Modification of Monogastric Digestive System***

Recent trends in consumer attitudes toward food producing animals, including pigs and poultry, are safe and better quality products from welfare applied animals (Adams 2006). Pigs and poultry do not have pregastric fermentation ability and therefore all feeds should be digested intestinally. Most of the developments in this area are related to the enzymes that ease the digestibility of certain feedstuff or break the cleavage of digestible nutrients with indigestible counterparts. Monogastrics lack certain enzymes that catalyze katabolic reactions. Therefore, technologically produced enzymes such as beta glucanase can be added to overcome these deficiencies. Additionally, probiotics are the other groups of substances have future in poultry industry (Smulikowska 2006).

Poultry industry currently uses biotechnologically produced aminoacids, vitamins, organic acids, other solvents, and enzymes (Demain 2007). After glutamate (used as flavor enhancer), lysine, methionine, tryptophan, and treonine are produced in large amounts each year for the purpose of poultry diet supplementation. Additionally, chemical production of vitamin production facilitates switching their production toward fermentation processes, which involve a few bacteria and exchange genes to produce more effectively (Demain 2007). Of that produced almost half the vitamins are used for the animal feed industry and 16 % of the enzymes are used in agriculture and livestock industry (Demain 2007).

The purpose of supplying enzymes, probiotics, prebiotics, and synbiotics to poultry is to increase the animal's own supply, reduce the hazardous effects of antinutritive factors, increase the availability and absorption of certain nutrients, and modify the GI tract population (Ferket et al. 2005; Bonneau and Laarveld 1999). Although most poultry enterprises heavily depend on corn-soy-based diets, availability of other energy sources and grains and other oilseed and protein sources necessitate the use of commercially available, technologically produced enzymes, and other products. When maize is substituted with wheat or barley, endo-beta-1-4-xylanases and beta 1-3, 1-4 glucanases are used in poultry diet, otherwise “viscous grains” prevent absorption and availability of nutrients in the intestine (Cowieson et al. 2006). The purpose of addition of these enzymes is to breakdown arabinoxylans and beta glucans, which are called nonstarch polysaccharides (NSP) together with some others. If they are not supplemented, feed conversion ratio (FCR) will be poor, slow body weight gain (BWG), and sticky droppings especially in young chicks will be observed in broilers and laying hens (Bhat 2000). Additionally, clean eggs, better yolk color, uniform animals, reduced environmental waste, and increased digestion of other nutrients may be observed by the addition of single or multi-enzymes.

Enzyme amount and proper ratio may be adjusted in diets of poultry depending on the rate of use of these grains. FCR was improved by 2–9 %, BWG was improved by 4–12 %, and apparent metabolizable energy (AME) was improved by 3–10 % by the addition of xylanase and glucanase to barley, wheat, corn, rye, and triticale-based diets in broilers, ducks, turkeys, and laying hens (Cowieson et al. 2006). Multi-blend enzymes also modify the intestinal flora resulting in pathogen reduction and the non-pathogen downside effect on digestion, absorption, and metabolism and therefore improved FCR by up to 8.6 % and BWG by up to 17 % (Ferket et al. 2005). Currently, the use of relatively new by-product feedstuff such as dried distiller grains soluble (DDGS) and others may urge the supplementation of various blends of enzymes in poultry and pig diets (Cowieson et al. 2006).

Approximately 60–65 % of phosphorus in cereal grain is bound with phytic acid (Walsh et al. 1993). Presence of phytate in GI tract of birds may stimulate endogenous secretion, including mucin and, therefore, limit the digestibility of other nutrients. Addition of phytase to balanced poultry ration may increase amino acid absorption and retain more energy besides P retention to reduce environmental pollution (Cowieson et al. 2006).

After public concern and ban of antibiotic use in feeds, probiotics attracted more popularity among poultry nutritionists which is considered to decrease pathogen shedding in every aspect of production (Gaggia et al. 2010). With the concept of competitive exclusion, probiotics may have effects against common pathogens and the origin of food-borne illnesses such as *Salmonella* spp., pathogenic strains of *E.coli*, *Campylobacter jejuni*, *Clostridium perfringens*, and *Listeria monocytogenes* (Gaggia et al. 2010). Although few of the culturable species can be used as probiotics, they have relatively short shelf life and their survivability is most of the time questioned during feed processing and their passage through the digestive system with various pH environments such as low pH in gizzard (Ferket et al. 2005), *Lactobacilli* spp., *Bifidobacteria* spp., *Enterococcus faecium* have proved to be successful in eliminating pathogens in the GI tract and therefore improve performance during the early ages of broilers, possibly by affecting gene expression (Gaggia et al. 2010). Similarly, some of the egg production parameters were improved by addition of a mixed culture of probiotic microorganisms (Kurtoglu et al. 2004). There are attempts to increase the efficacy of probiotic microorganisms by modifying some of their genes; however, inconsistent outcomes for improvement in performance and health has been observed (Smulikowska 2006).

Currently, prebiotics are only from oligosaccharides with a degree of polymerization and are considered to be indigestible by animal enzymes in the upper portion of the GI tract (Saad et al. 2012). These indigestible oligosaccharides are commonly found in human and animal diets, however, their selectivity of nonpathogenic bacteria is still questionable (Gaggia et al. 2010). Prebiotics have several advantages over probiotics in that they are more stable during feed manufacture, do not require culturing, and are inexpensive to be produced (Ferket et al. 2005). Currently, Mannan-oligosaccharides (MOS), Fructo-Oligosaccharides (FOS), Inulin, Trans-Galacto-Oligosaccharides (TGOS), and Xylo-Oligosaccharides (XOS) have



been used in human and animal studies for their potential for selectively providing substrate for short chain fatty acid (SCFA) producing microbiota in the intestines (Saad et al. 2012; Gaggia et al. 2010). Possible mode of action was attributed to their potential for stimulating lactic acid bacteria and SCFA in *seca* (Ferket et al. 2005). Bacterial and fungal organisms, which have FructosylTransferase (Ftase) enzyme, are capable of producing FOS from sucrose and, by using recombinant DNA technology, FOS can be produced in *Pichiapastoris* yeast or in *E. coli* carrying gene from *Lactobacillus reutri* (Sangeetha et al. 2005).

A mixture of probiotics and prebiotics, called synbiotics, was achieved by combining lactobacillus with lactose, FOS with *B. subtilis*, GOS with *Bifidobacteria* spp and *E. faecium*, and chicory prebiotics and they were proved to have potential for health and performance improvement (Gaggia et al. 2010).

Studies of biotechnology in pigs have been directed to the phytase activity to reduce P excretion, NSPase activity to increase nutrient availability, and porcine somatotropin (PST) to improve growth performance. Additionally, gene mapping of pigs for their potential as an organ donor is the major biotechnological investigation area. Probiotics and prebiotics commonly used for all monogastric species have been investigated and used extensively in pigs and *Lactobacillus* spp., *Bifidobacterium* spp., and *Saccharomyces cerevisea* are proved to be useful during diseases such as diarrhea, necrotic enteritis, and other pathogen-related GI tract diseases and during weaning of piglets (Gaggia et al. 2010).

## 2.3 Feedstuff Development Through Biotechnology

### 2.3.1 Increase in Abundance of Nutrient

Livestock production cannot be separated from other fields of agriculture since feeds are mostly plant origin. Plants have short generation cycle compared to animals and it is easier and cheaper to make genetic manipulations. Biotechnological applications in plants can be classified into two groups; the first type of applications were administered through disease or insect resistance of the plants; second, to improve the nutritional contents of the plants. Removing the antinutritive factors over the years helped nutritionists to get increased amount of nutrients in feedstuff for animals. In the last two decades, global biotech crop producing lands have been growing exponentially and occupies 66 % of the world's cropland (UN 2004). Using genetic engineering, the desired characteristics can be transferred from one organism to other, which cannot be achieved with conventional breeding techniques. Public concerns about animals that are fed with genetically modified (GM) or genetically engineered (GE) crops do not have a strong base, since those animals even if they do not eat any GM feed at all, may have enzymes, vaccines, hormones, probiotics, and prebiotics from GM sources (Novoselova et al. 2007). In 2003, transgenic crops for corn, soybean, cotton, and canola were 40, 81, 73, and



70 % of total production, respectively, in the US (Gavrilescu and Chisti 2005). In 2008, these numbers reached 90+, 87, and 90+ as an acreage for soybean, cotton, and canola respectively (Hartnell 2010).

Genetic coding of plants, animals, microorganisms, and many viruses are DNA and consist of 1000 or more nucleotides in plants and animals resulting in approximately 20 and  $50 \times 10^6$  different genes in a plant (Beever and Kemp 2000). Currently, genetic modification has been achieved in a way that mutation occurs and inserting the gene into a new organism has been done sequentially prior to adding to a bacteria containing Ti plasmid (Beever and Kemp 2000).

Production of crops for livestock feeds has been improved by genetically modifying crop seeds. The crops mostly modified are the energy and protein sources of animals. Major energy sources are grains including corn (maize), barley, wheat, rye, oats, and triticale. Of them, corn is modified to resist insects, to tolerate herbicides, to carry more starch, protein, certain aminoacids, fat, certain fatty acids, and finally to resist drought and harsh climatic conditions. The first GM crops commercialized for livestock feed in 1996 in the US were herbicide tolerant soybean and canola, pest protected cotton, and corn (Alexander et al. 2007). Since then the number of transgenic plants, planting area, and public concern for GE plants is increasing exponentially. Marker-assisted technology speed-up the process and made it easy to determine whether transferred gene is working; gene mapping made the trait selection appropriate and metabolomics allowed to determine the gene unacceptable in certain environment (Steering committee on global NRC 2008). Transgenic applications started with single traits, however, currently 2–3 traits crops are used extensively. Although most of the transgenic products are used in developed countries, transgenic rice was first used in Iran and largely used in China (Herd 2006). In human nutrition, attempts have been made to decrease undesirable properties of foods such as nonallergenic peanuts or hypocholesterol fats from corn and increase the desirable traits of plants such as high amino acid or protein materials or high sugar corn. With “Golden rice” which has high beta carotene content and the color is yellowish, more than 400 million people suffering from vitamin A deficiencies will be benefitted from transgenic manipulations (Herd 2006). Often, animal nutritionists do not seem to be interested in the agronomical traits of crops, however, environmental conditions force plant breeders to develop transgenic plants to produce in harsh environments. For example, drought has been one of the main concerns in recent years and traits are chosen to resist heat and produce in drought conditions or consume less water during growth (Federoff 2010).

Transgenic corn hybrids that are currently grown and used for grain feed and silage feed was reviewed by Owens (2005). Corn grain was in the beginning modified to be resistant to common corn insects such as corn borer or corn rootworm, and later modified for high nutrient contents, high digestibility, or certain industry needs such as waxy corn for the chocolate or food industry. In the same paper, relative feed values of transgenic versus “near-isogenic” or transgenic versus control corn hybrids were compared (Owens 2005). High starch, high protein, and high oil transgenic corns showed higher performance against isogenic or control

corn. Future prediction of the author is that fiber digestibility of corn silage, lowering the lignin content, increasing certain amino acids, fatty acids content, or increasing the bioavailability of minerals may be the aim of genetic engineering in corn plant.

### ***2.3.2 Increment in the Proportion of Certain Nutrient***

The term “functional foods” or “nutraceuticals” is used to determine the technology that satisfies the needs of organisms deficient in certain enzymes or needs more of a certain nutrient to keep them healthy or use special types of therapy without any problem with the help of nutraceuticals. Certain nutrients such as selenium can be enriched. Additionally, certain amino acids, fatty acids, sugars, and enzymes can be modified (Deynze et al. 2008). Decreasing the lignin contents of crops and increasing the NDF digestibility of corn and other forage-related crops is currently underway (Owens 2005).

Nutrition versus functionality of human foods was reviewed by McGloughlin (2010) and besides macronutrients micronutrients such as phytochemicals and antinutrients such as allergens and toxins have been investigated and those genes which are responsible for them have been modified to obtain functional foods.

## **2.4 In Vitro Gene Technologies Help Nutrition**

Enzymes manufactured in certain microorganisms can help in digestion of specific nutrients. Biotechnology-related products have been investigated and approved by the regulating authorities regionally or nationally in individual countries, and the last figure was more than 75 industrial enzymes used in various industries produced using biotechnology (UN 2004). Many microorganisms exchange genes to produce one or more enzymes to be used exogenously in animal nutrition. While exogenous enzymes are widely used in the poultry industry, enzymes in ruminant animals have been directed only at polysaccharidases (Beauchemin et al. 2006). The industry has used microorganisms to produce enzymes, ferment foods and feeds, and treat to reduce waste traditionally. However, rapid expansion of the enzyme industry led to production of 20,000 tons in 2005 and an expected expansion of 5–10 % each year. For example, *Trichoderma* produced cellulose can be used in the textile and feed industries (Gavrilescu and Chisti 2005). In vitro methods of production are cheaper, time saving, and have highly controllable and adjustable processes. In vitro produced enzymes can be added to animal feed just prior to feeding, however, their efficiency in various environments such as low pH and high temperature should be closely monitored.

## 2.5 Technologies to Reduce Animal Waste

Reducing animal waste is discussed in another section of this book, however, since it is related to nutrition and certain nutrients, this topic has been the major interest of nutritionists. Choosing high protein digestibility crops as animal feed will reduce wastage of nitrogen and will not pollute the underground water. Phytase enzyme breaks down the linkages between phosphorus and phytin, which avoids phosphorus from being digested. Phosphorus release into the environment poses threat to lands that have shallow underground water drainage. Methane was thought to be only loss of energy by the ruminant animal, however, it is considered as one of the major contributors to greenhouse emission and thus reducing methane through biotechnologically engineered feeds and rumen microorganism is currently being investigated.

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