Potential of Biotechnology for Livestock Feed Improvement: A review

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Abstract: *Application of recent advancements of biotechnology in recent years allowed the use of non-toxic fungi to improve fibrous feeds like straw or poor quality roughages. In particular, the white rot fungi have been used because of their ability to digest in to usable product of the plant material. In addition, wide variety of feed additives, many of biotechnological origin are known to modify rumen fermentation in ruminant animals. They include components that can reduce methanogenesis, enhance propionic acid production, reduce protein degradation, improve microbial protein synthesis and inhibit protozoa. Among such additives are antibiotics, probiotics and specific substrates like pre-biotics. Moreover, due to advances in biotechnology, more effective enzyme preparations can now be produced in large quantities and relatively inexpensively. Therefore, supplementation of the diet as a means of improving nutritive value is becoming routine. Still another area of application of biotechnology is reduction of less important microbes from rumen and increase feed efficiency of animals. The ultimate goal of using biotechnology in animal 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.*

Key words: Feed Additives, Feed Nutritive Improvement, Forage Breeding, Antibiotics, Enzymes, Probiotics and Prebiotics.

1. Introduction

Demand for livestock products is increasing because of the increasing human population, growth in income and urbanization (Thornton, 2010) in these parts of the globe. For example, total meat production in the developing world tripled between 1980 and 2002, from 45 to 134 million tons (World Bank, 2009). Demand for meat will grow only 0.6% in developed countries compared with an annual increase of 2.8% in developing countries. Most food of animal origin consumed in developing countries is currently supplied by small-scale, often mixed crop-livestock family farms or by pastoral livestock keepers (John and Maria, 2001). Hence, productivity of animals 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 (John and Maria, 2001). Conventional methods of livestock improvement have been used in the past served the purpose of increasing livestock productivity (Madan, 2005). However, these options can no longer sustain production; consequently, new intensive techniques including biotechnology are now required to augment productivity. 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 (Mahesh and Madhu, 2013). Hence, the objective(s) of this review article is therefore to indicate the potential application of biotechnology in animal feed improvement in developing countries.

2. Fibrous Feed Improvement

Fibrous feeds of low digestibility comprise the major proportion of feeds accessible to most ruminants under smallholder situations in developing countries (Lebbie and Kagwini, 1996). It is well known that some micro-organisms, including cellulose enzymes from anaerobic bacteria and white rot fungi (*Pleurotus ostreatus*) can degrade lignin in the cell walls. Several fungal strains have been used for lignocellulosic hydrolysis such as *Asprigullus niger, A. terreus, Fusarium moniliforme and Chaetomium celluloyticum* (Kim *et al*., 1985; Abdel-Azim *et al*, 2011). However, among many species of fungi white rot fungi have been reported to be suitable for treatment of roughages so far. Zadrazil *et al*. (1995) found that, the white rot fungi have the capacity to attack lignin polymers, open aromatic rings and release low molecular weight fragments. In addition, Yu *et al.* (2009) have tested several such strains of white-rot fungi on sawdust and found that digestibility of the sawdust in vitro was improved, and the degree of improvement was highly correlated with the degree of lignin removed. In related work, Kirk and Moore (1972) found two strains, which increased the digestibility in vitro of sawdust from 46 to 74% in a period of 2–3 months. The sawdust lost 20% of its original weight and 50% and 20% of its original lignin and carbohydrates, respectively. These results are certainly encouraging and experiments using these organisms on straw should be taken up. Significant results were reported by Akinfemi *et al*. (2009) for CP (14%) 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 (Leng, 1991). In general, the organisms that suit for this purpose must have a number of special properties. 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 (Mahesh and Madhu, 2013). Another indirect approach to the enhancement of fibre digestion in ruminants is through modification of silage inoculants. 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 reviewed by Mahesh and Madhu (2013). 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. Scheirlinck *et al*. (1990) reported that recombinant *Lactobacillus plantarum*, a species used as silage starter, were constructed to express alpha-amylase, and cellulase or xylanase genes. The competitive growth and survival of such modified lactobacilli in silage has been reported by other workers (Sharp *et al*., 1992), although the impact on silage digestibility has not been studied.

3. Forage Breeding

Genetically engineered forage crops, with a range of potential benefits for production, the environment and human health, have been developed (Spangenberg *et al*. 2001). 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. These transgenic forage crops are aimed at offering a range of benefits to consumers, as well as developers and producers. 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 available set of transgenic food crops (Spangenberg *et al*. 2001).

Protein content and feeding quality are being targeted for improvement in biotech crops (Edwards *et. al*., 2000).. Scientists can modify the protein content of crops either indirectly (by improving nitrogen assimilation) or directly (by modifying key biochemical pathways or introducing proteins with a different amino acid composition). Researchers by elevating the levels of sulfur-containing amino acids in lupins, improved the performance of broiler chickens (Ravindran *et al*. 2008) as well as wool growth and weight gain in sheep.

High lignin content reduces the efficiency of feed utilization and thereby reduces animal growth. Conventionally breed forage varieties with reduced lignin are available, but they tend to have weaker stems and poor stand ability in the field. Researchers have developed engineered alfalfa with 20 percent less lignin and 10 percent more cellulose, a combination that makes it more digestible (Madan M.L., 2005). 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. It is known that forage legumes are comparatively low in sulphur-containing amino acids and their availability to ruminants is further adversely affected during rumen digestion (Croissant *et. al.*, 1976). This leads to the reduction of the optimum for animal growth level of essential amino acids. Plant genetic modification with genes encoding for a sulphur amino acid-rich proteins, resistant to rapid rumen degradation can compensate this deficiency. Agronomic researchers around the globe are currently using recombinant DNA technology to create new and altered species of plants.

Leng (1991) indicated that plants in order to survive insect, fungal and bacterial attack have developed secondary compounds, which detract from these organisms colonizing the leaf tissues. In another study, researchers at the Noble Foundation have been successful in manipulating lignin composition and levels in alfalfa and other forages to improve their digestibility and the conversion of biomass to biofuels (Chen and Dixon, 2007). Some shrubs and trees respond to leaf damage as occurs by grazing and produce greater quantities of secondary compounds that often make them inedible. Using biotechnology, scientists may be able to enhance the oil content of crops where there is no natural variation for this trait. High-oil corn reduces the amount of feed required for a livestock diet, and this in turn reduces the volume of manure (Etherton *et al*., 2003). 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 (Wieczorek , 2003).

Evidence suggests that genetically modified crops are more acceptable if they provide benefits for the consumer or the environment. For example, research indicated medical applications of genetic engineering are more acceptable to the public than food applications (Small et al, 2002). However, unlike genetically engineered food, forage crops are not eaten by people, rather, they are eaten by food animals; animals, the products of which (e.g., milk and meat), humans consume. In addition to consumer benefits, animal forages like soybeans, corn, canola, and cotton modified for agronomic input traits such as herbicide tolerance and insect protection are all used in livestock rations. They are present either as a whole crop such as corn silage, or as specific crop components or co-products such as corn grain or oilseed meals. Studies by Phipps *et al*. (2005) showed that the inclusion of genetically modified feed ingredients in dairy cow diets did not affect feed intake or milk production.

4. Feed Additives

Feed additives are materials that are administered to the animal to enhance the effectiveness of nutrients and exert their effects in the gut (Fuller, 2004). Feed additives include antibiotic, enzymes propbiotics and prebiotics (McDonald *et.al*. 2010).

Antibiotics

Antibiotics are antimicrobial pharmaceutical, usually of plant or fungal origin and are also synthesized in the laboratory (Fuller, 2004). Although the primary use of antibiotics is in the treatment of infections, certain antibiotics are used as feed additives in order to improve growth and feed conversion efficiency. Among antibiotic groups are ionophores (McDonald *et al*., 2010) which are ion-bearing compounds, which surrounds cations so that the hydrophilic ion can be shuttled across hydrophobic cellular membranes to defeat the normal concentration gradient essential in living cells (Fuller, 2004). For example, valinomycin is a cyclic peptide which binds potassium, while monensin is a carboxylic ionophore which displays a binding preference for sodium. Ionophores are used in ruminant animals like cattle to improve feed efficiency by shifting rumen fermentation towards the production of more propionic acid, which can be used by the animal and less methane, which is lost. Ionophores hereby change the pattern of rumen microorganisms, reducing the production of acetate, butyrate and methane, and increasing the proportion of propionate (McDonald *et al*., 2010). Since methane is a waste product, the efficiency of rumen activity is improved. Ionophores also reduce the total mass of bacteria and thereby decrease the amount of dietary protein degraded. Avilomycin is licensed for use in pigs, broiler chickens and turkeys. Salinomycin is an ionophore available for use in pigs and also used to prevent coccidiosis in broiler chickens (Fuller, 2004).

McGuffey *et al*. (2001) also reviewed that ionophores have general metabolic role within the animal through improving production efficiency by providing a competitive advantage for certain microbes at the expense of others. In general, the metabolism of the selected microorganisms favors the host animal. In another report, broilers receiving the diet supplemented with antibiotic had significantly lower total aerobic bacterial counts in the small intestines compared to those on the other dietary treatments (Sarica *et al*., 2005). The combined supplementation of the antibiotic and enzyme resulted in a significantly lower *Escherichia coli* concentration in the small intestines compared to the basal diet and the other dietary treatments.

Enzymes

As a result of advances in biotechnology, more effective enzyme preparations can now be produced in large quantities and relatively inexpensively (McDonald *et al*., 2010). Therefore, supplementation of the diet as a means of improving nutritive value is becoming commonplace. The enzymes used as food additives act in a number of ways. According to Fuller (2004), enzymes are mainly used in the diets of non-ruminants but are also added to ruminant diets. Their main purpose is to improve the nutritive value of diets, especially when poor-quality, and usually less expensive, ingredients are incorporated. Common example of enzymes is use of phytase feed enzyme in monogastric diets. Phytase feed enzymes have more general application as their substrate is invariably present in pig and poultry diets and their dietary inclusion economically generates bio-available phosphorous and reduces the phosphorous load on the environment. The prohibition of protein meals of animal origin, which also provide phosphorous, has accelerated the acceptance of phytase feed enzymes in certain countries (Fuller, 2004).

Amino acid digestibility may also be improved with phytase supplementation. In a study with finishing pigs, Zhang and Kornegay (1999) reported that the digestibility of all amino acids except proline and glycine increased linearly as phytase supplementation increased. In ruminant nutrition, enzymes improve the availability of plant storage polysaccharides (e.g. starch), oils and proteins, which are protected from digestive enzymes by the impermeable cell wall structures. Thus, cellulases can be used to break down cellulose, which is not degraded by endogenous mammalian enzymes. Enzymes are essential for the breakdown of cell-wall carbohydrates to release the sugars necessary for the growth of the lactic acid bacteria. Although resident plant-enzymes and acid hydrolysis produce simple sugars from these carbohydrates, addition of enzymes derived from certain bacteria, e.g. *Aspergillus niger* or *Trichoderma viridi* (Parawira and Esther,2008) increases the amount of available sugars. Commercial hemicellulase and cellulase enzyme cocktails are now available and improve the fermentation process considerably (Hooper *et. al*., 1989) . However, prices of these products preclude their viability for farm level application, especially in developing countries.

Supplementation of a wheat by-product diet with cellulase increased the ileal digestibility of non-starch polysaccharides from 0.192 to 0.359 and crude protein from 0.65 to 0.71(McDonald *et al*., 2010). Typically, these enzymes fall into the general classification of cellulases or xylanases (Renaville and Burny, 2001). However, most commercial preparations are not single gene products, containing a single enzyme activity. The diversity of enzyme activities within commercially available enzyme preparations is probably advantageous, in that a single product can target a wide variety of substrates Renaville and Burny (2001).

Probiotics and prebiotics

Probiotics are feed supplements that are added to the diet of farm animals to improve intestinal microbial balance (Fuller, 2004). In contrast to the use of antibiotics as nutritional modifiers, which destroy bacteria, the inclusion of probiotics in foods is designed to encourage certain strains of bacteria in the gut at the expense of less desirable ones (McDonald *et. al*.,2010). Besides, these microorganisms are responsible for production of vitamins of the B complex and digestive enzymes, and for stimulation of intestinal mucosa immunity, increasing protection against toxins produced by pathogenic microorganisms. In ruminants, they are more effective in controlling the diseases of the gastrointestinal tract of young animals, as there is no complication of the rumenmicro-flora. The initial colonization of the small intestine is from the dam's microflora and the immediate surroundings, and usually includes streptococci, *E. coli* and *Clostridium welchii*. When milk feeding commences, the lactobacilli become the predominant bacteria present. Calf probiotics contain benign lactobacilli or streptococci and are likely to be valuable only when given to calves that have suffered stress or have been treated with antibiotics that have destroyed the natural microflora (Fuller, 2004). Addition of probiotics to the diet produces variable benefit, depending on whether the animals are in poor health. It is also difficult to determine which bacterial species would be beneficial in any given circumstance. Probiotics have sometimes been found to be beneficial in protecting pigs from infectious diseases. Lactic acid bacteria isolated from the gastrointestinal tract of pigs, such as *Enterococcus faecium* and *L. acidophilus*, can inhibit enteric indicator strains, such as *Salmonella enteritidis*, *S. cholera suis*, *S. typhimurium* and *Yersinia enterocolitica*. Dry yeast (*Saccharomyces cerevisiae*) has the advantage over bacterial probiotics that it is more tolerant of extreme pH and environmental conditions. Probiotic use is subject to extensive legislation designed to protect farm animals and consumers. In adult ruminants, yeasts may be used as probiotics to improve rumen fermentation (Fuller, 2004).

Prebiotics are defined as non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and activity of one or a limited number of bacteria in the colon (Gibson and Roberfroid, 1995). The most common prebiotics are oligosaccharides, which are non-digestible carbohydrates. The way in which prebiotics act is by (1) supplying nutrients to beneficial microbes, or (2) tricking pathogenic bacteria into attaching to the oligosaccharide rather than to the intestinal mucosa. This reduces the intestinal colonization thereby decreasing the incidence of infection in the birds. Because the oligosaccharide is non-digestible, the microbes that are attached will travel along the gastro-intestinal tract with the ingesta, and are excreted from the bird along with other undigested food.

Live microbial cultures and their extracts, particularly of *Aspergillus oryzae* and *Saccharomyces cereuisiae,* have been used as feed additives for many years. Their widespread use as manipulating agents for ruminal fermentation, so called direct-fed microbials, is more recent, as are most of the research papers (Wallace and Newbold, 1992). The improved feed intake seems to be driven partly by an improved rate of fiber breakdown and partly by an improved duodenal flow of absorbable amino-nitrogen. These two observations are suggested to arise from a more active microbial population: the most reproducible effect of microbial feed additives is that they increase the viable count of anaerobic bacteria recovered from ruminal fluid. Increases of 50 to 100% are common (Wallace and Newbold, 1993), but increases of more than 10-fold compared with controls have been observed. Cellulolytic bacterial numbers are increased (Wallace and Newbold, 1993) and lactic acidutilizing bacteria are stimulated by the dicarboxylic acids present, thus explaining in part the improvement in fiber breakdown and increased stability of the fermentation in animals receiving yeast and *A. oryzae*. Mehdi *et al*. (2011) reported that dietary inclusion of probiotic and prebiotic supported a superior performance of chicks and can be applied as antibiotic growth promoter substitutions in broilers diet.

5. Defaunaion

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 (Wael *et. al*., 1998). 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 (McDonald *et al*., 2010). However, the rumen methanogenic micro-organisms could also be sensitive because of their archaebacteriai cell nature and loss of these hydrogen-gas-utilizing methanogenic organisms would drastically disrupt the entire rumen fermentation system. The metabolism of other bacterial species would also have to be genetically engineered to provide a hydrogen sink. One possibility would be to engineer *Eubacterium limosum*, a relatively numerically minor species in the rumen, preferentiallyto form acetate and butyrate from HP and carbon dioxide.

In another study (Hsu, 1991), defaunation did not decrease total free amino acid concentrations in ruminal fluid, but it altered the profile of free amino acids. Although defaunation increased ruminal bacterial numbers, no increases in total microbial crude protein or organic matter concentrations in ruminal contents were observed. Diaz *et al.* (1993) reported that for sheep based forage rudediets as protozoal population reduced (84%), the degradability of the dry matter at 24 h also increased significantly. 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.

6. Conclusion

Biotechnology in animal production in developing countries has been applied only in a few areas such as conservation, animal improvement, healthcare and augmentation of feed resources. Poor quality feeds are the major bottleneck for livestock production and productivity. It could be concluded that there are several potential opportunities for improving the efficiency of ruminant digestion and possibilities for utilizing a wider range of feeds than is currently possible. The fibrous feeds, including crop residues, of low digestibility constitute the major proportion of feeds available to most livestock species of smallholder situations in developing countries. Although use of biotechnology in animal feed improvement is a new avenue in the developing world, there are still possibilities to improve fibrous feeds through biological treatments such as development of white rot fungi on fibrous feeds. Modifying forage resources is another option in which digestibility, amino acid composition and overall feed value is improved. Additives to animal nutrition, such as enzymes, probiotics, single-cell proteins and antibiotics in feed, are already widely used in intensive production systems worldwide to improve the nutrient availability of feeds and the productivity of livestock. It can be concluded that there are several potential opportunities for improving the efficiency utilizing a wider range of feeds than is currently possible. The microbial flora of the rumen can be successfully manipulated if such manipulations are adding exogenous fibrolytic enzymes to ruminants can potentially improve cell wall digestion and the efficiency of feed utilization. The use of biotechnology to improve post-ingestion quality of fibrous forages is on the verge of delivering practical benefits to ruminant production system. The microbial flora of the rumen can be successfully manipulated if such manipulations are adding exogenous fibrolytic enzymes to ruminants can potentially improve cell wall digestion and the efficiency of feed utilization.

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