

**RECENT ADVANCES IN THE APPLICATION OF BIOTECHNOLOGY IN
ANIMAL NUTRITION**

BY

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DECLARATION

I, Roland P. Varkpeh, do hereby declare that except for references cited to other people's work which have been duly acknowledged, this exercise is the result of my original research and that this long essay titled "**Recent Advances in the Application of Biotechnology in Animal Nutrition**" either in part or whole has not been presented elsewhere for the award of any degree.

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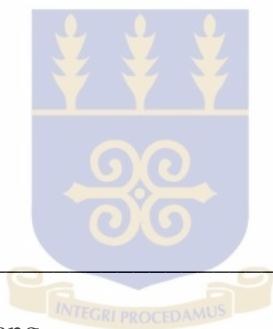
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I wish to thank God for making me strong throughout this process. I also thank God for my family for their prayers. Praise be to God for His many blessings.

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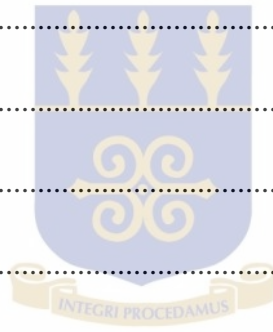
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ABSTRACT

Biotechnology is the use of biological processes, organisms, or systems to manufacture products intended to improve plants and animals for human use. Recently, there are wide potential applications of biotechnology in the field of animal production to increase the productivity of animals through better plane of nutrition, better production and improved health conditions. Hence this long essay was undertaken to review current techniques used in animal biotechnology with emphasis on animal nutrition and their contribution to livestock production and to recommend biotechnological advances in animal nutrition that should be adopted to boost livestock production in Africa. Nutrients like protein, amino acids and fats can be produced or protected according to the need at different physiological states of the animals. Enzymes can be used to improve the availability of nutrients from feed and to reduce the wastage of the feed and fodder. Prebiotics and probiotics or immune supplements can be useful to inhibit enteric pathogenic bacteria. Along with these, use of plant biotechnology to produce feed and fodder with good nutritive values can be done with ease. Furthermore, addition of vaccines or antibodies in feeds can be used to protect the animals from diseases. It is also possible to genetically manipulate rumen microbes to improve the health of animals. However, there are challenges in accepting the role of biotechnology in animal nutrition, which mainly depend on social and cultural norms and economic importance to consumers and society. Nevertheless, biotechnology is already gaining widespread use in animal production and there are numerous other potential applications. It is concluded that the application of biotechnology can be envisaged for improving the performance of animals through better nutrition, enhanced production potential or improved health status. Also, nutrients (i.e. amino acids) can be produced and/or protected, resulting in improved formulation of diets that more accurately meet specific needs for productive functions.

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CHAPTER ONE

1.0 INTRODUCTION

Animal agriculture has made great contributions to human development in the past century. Animal products provide one-sixth of human food energy and more than one-third of the protein on a global basis (Bradford, 2000). Further increases in per capita and total demand for animal food products are forecast for the globe, mainly developing countries for the next decades. However, animal agriculture is increasingly suffering challenges from environmental protection (Chen, 2001) and public health concerns (Barton and Hart, 2001). Future animal agriculture will be subject to more and more legal restrictions. One example is that all except four antibiotics and some animal-origin feeds such as meat and bone meal have been proscribed as animal feeds in the European Union. Forage crops or roughage are mainly imperative feed for ruminants. In general, forage crops have lower quality in the tropic than temperate zone depending on the kind, age and geology (Ramchurn and Raggoo, 2000). The amount of fibre in forage crops in the tropical zone is higher than that in the temperate zone, with digestibility in the tropics being less than that in the temperate zone (Herrera *et al.*, 2007). Protein and digestibility reduce as forage crops mature (Mohammed *et al.*, 2007). However, ruminants are able to utilise roughage because of their rumen microorganisms which produce enzymes to digest plant cell wall contents (cellulose and hemicelluloses) (Ando *et al.*, 2004).

Supraraksana and Soyong (2006) reported that a new strain of *Chaetomium globosum* could have cellulolytic and lignolytic activities that degrade organic substances. The improvement of roughage quality can therefore be achieved by introduction of microorganisms that produce such enzymes to enable the animal to maximally utilize the forage crops for their growth (Yami,

2007). As a result, the further development or even maintenance of current output of animal agriculture may become more difficult than ever by applying only known technologies. Therefore, new technologies are not only essential but extremely urgent.

The major feed resources available for ruminants particularly in arid and semi-arid areas are fibrous residues from cereal crops and forages from rangeland. Usually both sources of ruminant diets are low in nitrogen (N) and digestibility (Dong *et al.*, 2003). The nutritive value of the feed consumed and the efficiency with which rumen microorganisms convert dietary carbohydrate to end product of fermentation, i.e. Volatile Fatty Acids (VFA), determine the nutrient supply to the ruminant (Tessendlo Group, 2004). For that reason, maximizing efficiency of breakdown and digestion of plant cell walls in the rumen can have a marked effect on animal productivity (Ghazanfar *et al.*, 2011). Several methods are currently employed to manipulate rumen fermentation to enhance post-ingestion nutritive value of fibrous forages through use of biotechnology including inoculants of native and recombinant rumen microorganisms, natural adaptation and microbial feed enzymes (Flint and Scott, 2000). In this essay, progress and problems related to manipulation of rumen ecosystem through inoculation of natural and genetically modified rumen microorganisms will be discussed. Advancements in the use of exogenous microbial enzymes for increasing digestibility of fibrous diets will also be taken into account.

Biotechnology can be broadly defined as using organisms or their products for commercial purposes. As such, (traditional) biotechnology has been practiced since pre-historic times. It has been used to bake bread, brew alcoholic beverages, and breed food crops or domestic animals.

Biotechnology is important if the world is to respond to the increasing demand for more food from livestock due to population growth. In general, biotechnology in livestock production can be categorized into biological, chemical and physical techniques that influence animal health (survival), nutrition, breeding and reproduction (Ben Salem *et al.*, 2007). These techniques have been applied mostly in developed countries but their application in Africa is minimal due to reasons related to economic augmentation such as poor infrastructure, technical and educational capacity. However, Africa can still benefit from tailor-made technologies that abridge complex techniques into applicable form through strategic packaging. Nevertheless, public concerns on food safety, environment and ethics are issues that cannot be ignored (Foidl *et al.*, 2001).

In most developing countries, biotechnological applications relating to livestock need to be suitable for animal owners who are resource-poor small-scale operators who own little or no land and few animals. Livestock is becoming increasingly important to economic growth in developing countries and the application of biotechnology is largely dictated by commercial considerations and socio-economic goals. Using technology to support livestock production is an integral part of viable agriculture in multi-enterprise systems. Livestock are part of a fragile ecosystem and a rich source of animal biodiversity, as local species and breeds possess genes and traits of excellence. Biotechnology will provide new and unprecedented opportunities to improve the productivity of animals through increased growth, carcass quality and reproduction, improved nutrition and feed utilization, improved quality and safety of food, improved health and welfare of animals, and reduced waste through more efficient utilization of resources.

This review is intended to introduce the recent advances in the application of biotechnology in animal nutrition. In spite of various advances in developed countries, the traditional system of livestock rearing in developing countries remain unchanged because of poor socioeconomic

conditions, scarcity of feed and fodder, poor production potential of animals, poor health and management conditions of livestock (Krishna *et al.*, 1998). Due to improved breeding policy, the genetic makeup of animals has been improved but the major constraint is shortage of good quality feed and fodder to meet their balanced nutritional requirements. Hence, there is an urgent need to handle this deficiency of balanced nutrition of ruminants. The present article is an attempt to focus on the role of biotechnology in improving the nutritional base of livestock.

1.1 JUSTIFICATION

As a step towards food security in Africa, meat production is expected to increase considerably in 2020 (Pinstrup-Anderson *et al.*, 1999) to meet the demand caused by population growth and rising income. There is the need to look at the recent advances and application of biotechnology in the improvement of animal nutrition to boost livestock production.

1.2 OBJECTIVES

- i) Review current techniques used in animal biotechnology with emphasis on animal nutrition and their contribution to livestock production.
- ii) Recommend biotechnological advances in animal nutrition that should be adopted to boost livestock production in Africa.

CHAPTER TWO

2.0 ANIMAL NUTRITION

According to Jorge *et al.* (2007) biotechnology techniques have been widely used for legumes - important crops with excellent nutritional characteristics and soil improvement qualities. Limited work has been carried out with under-utilized legume crops such as velvet bean (*Mucuna* spp.), which have great potential for multiple uses. In recent decades, there has been increased interest in the potential of *Mucuna* species as a cover crop and green manure for tropical and subtropical regions. Bangani *et al.* (2000) reported that *Mucuna* is also used as a minor food crop in many countries and interest in it as livestock feed is growing. Other minor uses exist, such as roasting the seeds as a coffee substitute. More importantly, L-dopa, extracted from *Mucuna* bean seeds and plants, is used for symptomatic relief of Parkinson's disease (Dove, 2002). Despite these numerous qualities, some constraints have limited its adoption. Biotechnology techniques can provide a window of opportunity for new or expanding products of *Mucuna*. Earlier biotechnology work with *Mucuna* was mostly related to its medicinal uses and focused on the mechanism of L-dopa production. More recently, biotechnology has also been applied to identify the major virus diseases affecting *Mucuna*, to develop new diagnostic methods for early virus indexing of *in vitro* plants and to clean virus diseases using meristem and thermotherapy techniques, as well as to study genetic diversity through the use of molecular tools (Giddings *et al.*, 2000). There are still niches to be explored such as the numerous phytochemical qualities of *Mucuna* that can be used to benefit human and animal nutrition and health as well as the environment through use of these compounds in natural weed and pest control management. Somasiri *et al.* (2010) showed that, modern biotechnology involves molecular techniques that use whole or parts of living organisms to produce or improve commercial products and processes. It is a relatively new and rapidly evolving branch of molecular biology, which started

with the creation of the first recombinant gene 30 years ago. The application of biotechnology methods in the food and agricultural industry is one of the many aspects of biotechnology that has great impact on society. By the year 2050, it is expected that more than 10 billion people will be living on this planet, and it is also believed that there may not be enough resources to feed the world population (UNFPA, 2000). Advances in genetic engineering are revolutionizing the way we produce and consume food, and it is quite possible that in the next decade a large percentage of the food we eat will be bioengineered. This improvement can be achieved through the introduction of genes that encode for enzymes in the biosynthetic pathway of vitamins, essential amino acids, essential elements, and micronutrient binding proteins (Bertin and Andrieu, 2005). Nutrition represents one of the most serious limitations to livestock production in developing countries, especially in the tropics. Feed resources are inadequate in both quality and quantity, particularly during the dry seasons. Biotechnological options are available for improving rumen fermentation and enhancing the nutritive value and utilisation of agro-industrial by-products and other forages (Vasil, 2003).

Fibrous feeds, including crop residues, of low digestibility constitute the major proportion of feeds available to most ruminants under smallholder situations in developing countries. The associated low productivity can be overcome to some extent by several means, among which are: balancing of nutrients for the growth of rumen microflora thereby facilitating efficient fermentative digestion and providing small quantities of by-pass nutrients to balance the products of fermentative digestion, enhancing digestibility of fibrous feeds through treatment with alkali or by manipulating the balance of organisms in the rumen and genetic manipulation of rumen micro-organisms, currently acknowledged as potentially the most powerful tool for enhancing the rate and extent of digestion of low quality feeds (Sharma *et al.*, 2002). Rumen micro-

organisms can also be manipulated by adding antibiotics as feed additives, fats to eliminate or reduce rumen ciliate protozoa (defaunation), protein degradation protectors, methane inhibitors, buffer substances, bacteria or rumen content and/or branched chain volatile fatty acids (Taylor and Hefle, 2002).

2.1 BIOTECHNOLOGY IN ANIMAL NUTRITION

The largest impact of biotechnology on livestock production is increasing the livestock feeds through improving nutrient content as well as the digestibility of low quality feeds through use of efficient feed additives. Biotechnology is already widely used in animal production and there are numerous other potential applications. Application of biotechnology can be envisaged for improving the performance of animals through better nutrition, enhanced production potential or improved health status. Nutrients (amino acids) can be produced and/or protected, resulting in improved formulation of diets that more accurately meet specific needs for productive functions (Kleter *et al.*, 2001). Enzymes can improve the nutrient availability from feedstuffs, lower feed costs and reduce output of waste into the environment. Pre- and pro-biotics or immune supplements can inhibit pathogenic gut microorganisms or make the animal more resistant to them. Plant biotechnology can produce crops with improved nutritional value or incorporate vaccines or antibodies into feeds that will cheaply and effectively protect the animals against diseases. Transgenic manipulation of commensal gut or rumen microorganisms has considerable potential for improving nutrition, gut development and health in animals (Bourn *et al.*, 2001).

2.2 NUTRITION AND FEED UTILISATION

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. Aids 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 (Hristov *et al.*, 2000). Gene-based technologies are being increasingly used to improve animal nutrition, either through modifying the feeds to make them more digestible or through modifying the digestive and metabolic systems of the animals to enable them to make better use of the available feeds (Bedford, 2000). Feeds derived from genetically modified plants (a quarter of which are now grown in developing countries), such as grain, silage and hay, have contributed to increases in growth rates and milk yield. Genetically modified crops with improved amino acid profiles can be used to decrease nitrogen excretion in pigs and poultry. Increasing the levels of amino acids in grain means that the essential amino acid requirements of pigs and poultry can be met by diets that are lower in protein. According to Craig (2001) poultry feeding studies have been conducted with *biotech* corn and soybeans. The results showed that, performance of broilers and layers fed the agronomic-trait *biotech* crops were identical to the performance of birds fed the varieties or hybrids from which the agronomic-trait *biotech* crop was developed. In other words, the introduction of an agronomic gene results in a feedstuff that is nutritionally equivalent to the same hybrid or variety without the gene. According to Schire and Tamminga (2002), the evaluation of new biotechnologies in livestock production is often done with reductionist, biological criteria such as live-weight gain or feed efficiency, and in isolated, artificial and homogeneous experimental conditions. Too often, the recommendation to apply a specific technology, tested under experimental conditions, assumes that the introduction will not affect other parts in the system. Looking at the animal, farm and farming community as a system provides a more comprehensive framework for assessing the potentials and risks of applying biotechnology in animal nutrition (Sodeinde *et al.*, 2007). A high demand for food and restricted access of many poor farmers to external inputs leaves the production of animal feeds

often at a low priority for farmers in many developing countries. Particularly on the better soils and in densely populated areas, the production of cash and food crops is a first priority. As a result, poor quality crop residues such as straw and rangeland grasses are left as major sources of animal feed (Olaniyi, 2006). Limitations for animal production in developing countries are, therefore, the lack of feed in terms of quality, quantity and seasonal availability. The lack of quality of ruminant feeds is caused by a high lignin content, low digestible cell walls in crop residues and mature grasses, usually associated with a low content of nitrogen (N), phosphorus (P) and sulphur (S). Attempts to use biotechnology through pre-fermentation of straws with aerobic fungi have not been successful (Somasiri *et al.*, 2010). The digestibility could be improved in some cases, but almost invariably at the expense of organic matter losses, costly process control and at the risk of mycotoxin formation due to contaminant organisms (Akter *et al.*, 2004). Possible alternatives to this approach could be the pre-treatment of fibrous feeds with enzymes, such as cellulases. Such enzymes are abundantly present in the rumen but they are cell-bound and they tend to be too large to penetrate pores in the cell walls. In other words, they are not very effective in breaking down the cell wall. (Somasiri *et al.* 2010) reported that fungal cellulases usually do not associate in large complexes, but are secreted freely in the medium. If the transit through the digestive tract is too fast, or the rate of degradation too slow for the successful use of these feeds, fortified with enzymes, then the ensiling of fodder with fungal cellulases together with a stabilization period of weeks to months may be an option. The conservation of high quality feed by ensiling fodder or hay making also seems an option in situations with periodically sufficiently available high quality material. In temperate regions this has been a very successful way of maintaining quality, but the method is little used in developing countries. Possible technical explanations could be the difficulty to maintain anaerobic

conditions. Improvements seem possible by using additives (energy, N, P, and S), enzyme producers (microbes) or enzymes (cellulases) (Aye, 2007). However, this way of securing feed quality requires skills which may not always be available at the right moment. Also, the cost of ensilage is often higher than the expected benefits; particularly the cost of airtight cover material and additives may be prohibitive. By-product feeds for non-ruminants (pigs, poultry) in rural areas are often available in limited quantities, for example, rice bran or oil seed cakes. Quite often they are exported or sold to urban centres. Their protein and phosphorus (P) content seems adequate to support reasonable levels of animal production (Bamikole and Babayemi, 2004). However, a large proportion of the P is present in the compound *inositol*, a form of P not available to monogastric animals such as pigs. The addition of the enzyme *phytase* has proven to be successful in developed countries, and some scope could exist for developing countries as well (Bangani *et al*, 2000). Suphalucksana and Soyong (2006) showed that ascospores of *Chaetomium cupreum* AN102 growing in rice straw and para-grass, may be used to develop as biological animal feed for ruminants. The study revealed that sterilized rice straw and para-grass were rapidly degraded by applying the specific strain of *C. cupreum* for one month. The feed composition analyses revealed that *Chaetomium* treated with those organic substances gave the highest potential to degrade cellulose this was followed by hemicellulose and crude fibre, respectively, which significantly different when compared to the non-treated one. Moreover, *Chaetomium* treated in sterilized para-grass gave significantly higher nitrogen free extract (NFE) than non-sterilized para-grass which indicated that *C. cupreum* An 102 could be used to improve the ruminant nutrition through biodegradation.

According to Kahi and Rewe (2008) livestock production is expected to grow tremendously in line with the projected demand for animal products. Therefore, the methods of livestock

production must change to allow for efficiency and improvement in productivity. Animal nutrition has for a long time provided one of the greatest challenges to animal production with precincts arising from both quality and quantity. A large proportion of animal feeds are fibrous with varying levels of digestibility and nutritive values. Animal nutritionists have developed *biotechs* to improve nutritive value for feeds, enhance digestibility and acceptability and removal of anti-nutritive factors from feeds especially for ruminant animals (Foidl *et al.*, 2001). The methods currently used in the treatment of animal feeds to improve intake and digestibility are mainly biological, physical or chemical. Lignin has been identified as the main cause of difficult biotechnology digestion for fibrous material. A potent lignase enzyme produced by the soft-rot fungus (*Phanerochaete chrysosporium*) that causes a high degree of depolymerisation of lignin is now available (Sajjad *et al.*, 2008). Although the lignase levels produced by the fungi cannot meet the requirements for commercial treatments of straw, recombinant DNA technology has been tipped to have the potential to modify lignase genes and proteins to increase efficiency and stability considering that the lignin gene has already been cloned and sequenced from *P. chrysosporium* (Ziemer *et al.*, 2002). Supplementation of roughage diets is basically the method of choice for many farmers in improving the nutritive value of animal feeds. However, removal of anti-nutritive factors has become a subject of great debate.

Anti-nutritive factors in plants such as protease inhibitors, tannins, phytohaemagglutinins and cynogens mainly in legumes have been tackled via plant breeding to either reduce or eliminate them. Inactivation as well as detoxification of anti-nutritive factors can also be done using transgenic bacteria given the success of experimental results of SMEDA (2009) using caprine rumen inoculum in cattle to detoxify a breakdown product of non-protein amino acid mimosine found in *Leucaena* forage. Similarly, conserved feeds also suffer the risk of being of low

nutritive quality if poorly fermented. Sterile conditions are normally necessary to allow for rapid growth of lactic acid bacteria that influence the anaerobic fermentation of sugars. Additives such as chloroform, toluene and cresol are still being used to inhibit bacterial growth while appropriate pH levels are achieved through addition of acids like sulphuric, hydrochloric acids and formic acids. Obviously, the hazardous nature of acids has intensified the search for alternative compounds for fermentation that include molasses, enzymes and inoculi of lactic acid bacteria. Extensive reviews on the use of enzymes to improve the nutritive value of animal feeds are available (Bedford, 2000). Manipulation of digestive tract environments which includes mainly the rumen in ruminants and intestinal tract in monogastrics using prebiotics and probiotics has been reported to be beneficial and effective in increasing the availability of nutrients to the animal (Foidl *et al.*, 2001). The main focus in the rumen is the manipulation of microbial flora population and type. Although attempts have been made to introduce transgenic bacteria in the rumen environments to increase efficiency of rumen fermentation, the results have been unsatisfactory due to the complex nature of the rumen environment that include the internal competition of rumen microbes which limits availability of energy (Flint and Scott, 2000). The manipulation of the rumen environment to improve the nutritive value of fibrous feedstuffs has been extensively reviewed (Msweeney *et al.*, 2000) with suggestions and limitations for the use of genetically modified organisms (GMOs).

Animal nutrition *biotech* is steadily developing in Africa mostly through local technologies that ensure degradation of fibrous feedstuffs. However local technologies suffer from mechanical bottleneck thus making adoption tedious and impractical for in large scale scenarios (Denev, 2006). The availability of lignase enzyme produced by the soft-rot fungus (*Phanerochaete chrysosporium*) that causes a high degree of depolymerisation of lignin (Dawson, 2002) should

be embraced for application in large scale livestock production systems. As opposed to forage degradation, a more current problem is antinutritive factors that hinder acceptability and diminish the nutritive value of animal feeds. Although this constraint has been circumvented elsewhere through plant breeding and use of transgenic bacteria to eliminate, reduce or detoxify feeds, little has been done in Africa. Similarly, boosting the nutritive value of conserved feeds is of great concern in Africa due to seasonal variation in feed availability. The main treatment for silage is still primarily molasses as opposed to enzymes and inoculi of lactic acid bacteria (Formigoni *et al.*, 2005). The absence of large scale production of silage for commercial purposes in most sub-Saharan countries has limited the use of additives such as chloroform, toluene and cresol to inhibit bacterial growth in order to facilitate fermentation by lactic acid bacteria. Manipulation of the digestive tract environment, especially rumen microbial flora population and type is an efficient means of ensuring nutrient availability to animals. There seems to be an over concentration on treatment of feeds to ease digestibility, acceptability and improvement of nutritive value as opposed to preparation of the animal's system to uptake nutrients. The suggestions and limitation for the use of genetically modified organisms (GMOs) should be studied carefully to fully establish how well the digestive tract of animals, the rumen in particular, can be manipulated to increase availability of ingested nutrients to the animal (Flint and Scott, 2000)

2.3 BIOTECHNOLOGIES FOR THE GUT MICROORGANISMS

Biotechnology can be extensively used for improving metabolism and activity of gut microorganism, which is very important for animal health and growth. This can be done in three biotechnological approaches. One is the application of biotechnological products to improve the gut ecosystem and promote the growth of beneficial bacteria. Pre- or pro-biotics and

oligosaccharides belong to this category. The second approach is to genetically modify microorganisms naturally present in the gut to enhance their capacity of defined functions or to add new functions. Introductions of diverse genes into gut microorganisms have been extensively explored (McSweeney *et al.*, 2000). The genetically modified microorganisms are able either to digest fibrous components and lignins of forages, to degrade toxins, to synthesize essential amino acids, to reduce methane formation, or to tolerate acids. The third approach is to introduce new species or strains of microorganisms into the gut (Raeth-Knight *et al.*, 2007). This application has a great potential to increase digestibilities of feedstuffs and to improve animal health and growth. However, as with many other techniques, biotechnology for gut microorganisms is far from being commercially applicable because of the technical problems and public concerns (Ekinici *et al.*, 2002). Expression of modified resulted in more fibre digestion but could not effectively compete with native highly fibrolytic bacteria under the in vivo environmental condition of the rumen (Krause *et al.*, 2003). Kobayshi and Yamamoto (2002) investigated the factors that limit maintenance of recombinant bacterium (*Butyrivibrio fibrisolvens*) expressing a foreign xylanase gene (*B. fibrisolvens* NO) and found that depression in 4 survivability of the recombinant was mainly due to heatsensitive antibacterial factors associating with microbial cells in the rumen. Conversely, Ziemer *et al.* (2002) found that when a natural bacterium *Butyrivibrio thetaiotaomicron* was added to a rumen model, it increased the fibre digestion. Matin *et al.* (2001) found that the fibrolytic activity of *Fibrococcus mucinogens*, *Ruminococcus albus* and *Ruminococcus flavefaciens* was depressed with cereal supplementation and there was no modification of the balance of the three cellulolytic bacterial species examined. Krause *et al.* (2003) also found that re-establishment of either natural or recombinant ruminal bacteria in the rumen is both variable and unpredictable and there are even reports of the

disappearance of the inoculant from the rumen after very short time period. However, in the two last decades, the potential roles of specific microbial supplements have been better defined and there has been considerable interest in using preparations containing live microorganisms as feed supplements for ruminants (Dawson, 2002).

Probiotics are live microbial feed supplements which beneficially affect the host by improving its intestinal microbial balance (Ekinici *et al.*, 2002). Correspondingly, in feed regulation, probiotics are included in the group of feed additives for stabilising the microbial communities of the digestive tract in monogastric animals and ruminants. They are also known as digestive bioregulators or direct-fed microbials (DFMs). In a narrower sense, the term probiotics is confined to products which consist of one, or a few, well-defined strains of microorganisms. Historically, bacteria and yeasts have served man very well in agriculture and nutrition. Well-known examples are the use of bacteria (mainly lactic acid bacteria) for production of silage, fermented cabbage (sauerkraut) and sour milk products such as yoghurt, cottage cheese and kefir and the use of yeasts (mainly *Saccharomyces cerevisiae*) for production of bread, beer and wine. Systematic research into probiotics for human use began at the beginning of the 20th century. Elie Metchnikoff, a Russian biologist who worked around 1900 at the Institute Pasteur in Paris, studied the mystery of the high life expectancy of Cossacks in Bulgaria. He related their extraordinarily high life expectancy of 115 years and more to their very high consumption of fermented milk products. He named the microorganism relevant for the fermentation *Bacillus bulgaricus*, later classified as *Lactobacillus bulgaricus*, which was used against scours and gastrointestinal diseases in humans as early as the 1920s. There was little interest in probiotics during the following decades until the 1960s and 1970s when they were rediscovered for human and animal nutrition. The first potent products for animal nutrition to fulfill the specific

requirements for feed additives did not appear on the European market until the mid-1980s. Today, modern animal nutrition has at its disposal a whole range of defined strains of probiotics belonging to the groups of lactic acid bacteria, Bacillus spores and yeasts. The commercial use of probiotics in the livestock industry, especially poultry, is relatively new. Only within the last few years have research workers documented the results of incorporating probiotics into poultry diets (Hristov *et al.* 2000). Probiotics have been found to improve animal performance and statistically significant responses have been reported. There have also been reports of no benefit from the use of probiotics in poultry diets. With the already high cost of feed ingredients rising each year any slight advantage, may prove to be economically beneficial to the producer. Probiotics, by favoring colonization with a specific group of beneficial micro organisms, create an environment biased against undesirable organisms. The use of probiotics in commercial poultry production is still in its developmental stages. More research needs to be conducted to determine why results are sometimes so variable with probiotics in poultry feeds. These data would make it easier for producers to decide whether or not probiotics should be used and would be even more important in the future if governmental agencies should ever prohibit the use of sub-therapeutic levels of antibiotics.

2.4 GENETICALLY MODIFIED RUMINAL MICROORGANISMS

With regard to fibre degradation in the rumen, much effort has been expended in developing genetically modified bacteria that would have superior fibre-degrading abilities. The construction of genetically modified bacteria has proceeded under the assumption that the rumen microbiota does not produce the correct mixture of enzymes to maximize plant cell degradation. It is well established that the principal fibrolytic bacteria of rumen are *Ruminococcus* and *Fibrobacter*, but it is thought that they do not produce exocellulases that are active against crystalline cellulose, so

this adding this activity would make them more potent. Ruminal bacterial species such as *Butyrivibrio fibrisolvens* and *Prevotella ruminicola* are found widely in ruminant animals on varied diets and are found in significant numbers regardless of the ruminal environment. These species, therefore, are logical choices to introduce new or enhanced genetic material into the rumen (Selinger *et al.*, 2000).

Over 100 different genes encoding enzymes for fibre digestion have been identified and cloned from ruminal bacteria such as *Butyrivibrio fibrisolvens*, *Fibrobacter succinogenes*, *Prevotella ruminicola*, *Ruminococcus albus* and *Ruminococcus flavefaciens*. At least 30 genes from ruminal fungi have been isolated that encode cellulase, xylanases, mannanases and endoglucanases. Almost 50% of the fibrolytic genes cloned have been sequenced (Bowman and Sowell, 2003). These genes are of particular interest due to their powerful fibrolytic activity and ability to break down very resistant cell wall polymers. Two plasmid vectors developed for use in other Gram positive bacteria have been introduced into four different *Ruminococcus albus* strains by electroporation (Matin *et al.*, 2001) and an efficiency of 3×10^5 transformants/ μg was achieved with one of the plasmids. A low frequency of transfer of the broad host range plasmid was also achieved into *R. albus* by conjugation from *Bacillus thuringiensis* BT351 (Aminov *et al.*, 2000). Despite these encouraging developments there have been no reports of introduction of vectors into *R. flavefaciens* strains and no studies on gene inactivation or the expression of genes introduced into *Ruminococcus*. Similarly there are no reports of indigenous plasmids or of successful attempts at gene transfer into this important cellulolytic species (Flint and Scott, 2000). In addition, cellulase and xylanase genes from ruminal protozoa have been cloned and most of the fibrolytic genes cloned have also been sequenced (Wang *et al.*, 2001). Protein bioengineering has been used to increase the catalytic activity and substrate diversity of fibrolytic enzymes from

ruminant microbes. This has resulted in enzymes which with up to 10 times higher specific activity, changed pH and temperature optima and increased substrate binding activity than the enzymes from which they originated (Wang *et al.*, 2004) *Streptococcus bovis* is found to be tolerant to zero (0) and two (2) depressed rumen pH, unlike most cellulolytic bacterial species in the rumen. In addition, there are convenient gene transfer methods available for this species that make it a candidate as a host for the expression of genes from other organisms (Bowman and Sowell, 2003). Ekinici *et al.* (2002) were able to use a (1,3-1,4)- glucanase promoter found in *S. bovis* to express a cellulase gene from the anaerobic rumen fungus *Neocallimastix patriciarum* that is found in very low levels in the rumen and is important for the degradation of crystalline cellulose. The resulting enzyme product was active against a wide variety of cellulosic substrates. The advantages of using fungal enzymes are their stability to low pH and their high activity level. Wang *et al.* (2004) were successful in introducing a xylanase gene from the anaerobic ruminal fungus *Neocallimastix patriciarum* into *Butyrivibrio fibrisolvens* and achieving secretion of the enzyme. More recently, Krause *et al.* (2003) constructed a recombinant *Butyrivibrio fibrisolvens* that expressed a xylanase enzyme from the ruminal fungus *Neocallimastix patriciarum*. The recombinant *Butyrivibrio fibrisolvens* did have an increased ability to digest fibre, but it did not persist in the rumen for more than 22 days. Hence, the biggest difficulty is the ability to establish and maintain the new strain in the mixed rumen population and survival of new strains is not well understood (Krause *et al.*, 2001)

2.5 REINTRODUCTION OF NATURAL AND GENETICALLY MODIFIED MICROBES TO THE RUMEN

The ecology of the introduced strains has been overlooked to a large degree and the success of this technology may ultimately depend on a better understanding of factors that determine establishment in a complex microbial ecosystem, fermenting microorganisms (Durand and

Fonty, 2001). Survival and population density have introduced a defined genotype into a complex microbial ecosystem is probably more difficult than originally expected (Durand and Fonty, 2001). Expression of modified *Neocallimastix patriciarum* xylanase in *Butyrivibrio fibrisovens* resulted in more fibre digestion but could not effectively compete with native highly fibrolytic bacteria under the *in vivo* environmental condition of the rumen (Krause *et al.*, 2003). Kobayshi and Yamamoto (2002) investigated the factors that limit maintenance of recombinant bacterium (*Butyrivibrio fibrisolvens*) expressing a foreign xylanase gene (*B. fibrisolvens* NO) and found that depression in four survivability of the recombinant was mainly due to heat sensitive antibacterial factors associating with microbial cells in the rumen. Conversely, Ziemer *et al.* (2002) found that when a natural bacterium *Butyrivibrio thetaiotaomicron* was added to a rumen model, it increased the fibre digestion. Matin *et al.* (2001) found that the fibrolytic activity of *Fibrococcus mucinogens*, *Ruminococcus albus* and *Ruminococcus flavefaciens* was depressed with cereal supplementation and there was no modification of the balance of the three cellulytic bacterial species examined. Krause *et al.* (2003) found that re-establishment of either natural or recombinant ruminal bacteria in the rumen is both variable and unpredictable and there are even reports of the disappearance of the inoculant from the rumen after very short time period (Aye and Adegun, 2010). Furthermore, the probability of colonizing the rumen with a bacterium not originating from the rumen is low (Makkar, 2007). According to McSweeney *et al.* (2000) Rumen is determined by many factors, which are

- 1) Competition with indigenous microbes for substrates that are used for growth

- 2) Growth at a rate faster than the dilution rate of the rumen

- 3) Adaptation to and tolerance of the chemical and physical environment in the rumen

4) Capability to withstand engulfment by protozoa and,

5) Resistance to inhibition by bacteriocins and infection by bacteriophage consistent with the principles of microbial ecology. Also therefore, systemic research is needed to further explore these factors that determine what additional conditions are required to ensure survival of introduced ruminal microorganism strains. key factor in limiting cell wall (NDF) digestibility by ruminant animals. Noble Foundation Scientists have been working on biotech manipulation of the lignin pathway since the early 1990's (Dixon and Reddy, 2003). This work has been the foundation of the Reduced Lignin project at the CAI. "Gene knockout" is a biotech process whereby expression of a specific individual gene is greatly reduced.

CHAPTER THREE

3.0 IMPROVED EFFICIENCY OF PROTEIN UTILIZATION

In 2002 three groups came together to start an inter-institutional, interdisciplinary research program to use plant biotechnology to improve key alfalfa characteristics that affect forage quality and efficiency for use by dairy cows. The Consortium for Alfalfa Improvement (CAI) was formed by the U.S. Dairy Forage Research Center, the Samuel Roberts Noble Foundation and Forage Genetics bringing together acclaimed experts in plant biochemistry, molecular biology, plant breeding, agronomy and ruminant nutrition (Grabber *et al.*, 2004). Pioneer HiBred International joined the CAI in 2007 interface between animal and plant scientists; and public and private institutions recognise the complexity of the tasks at hand, and the challenges of translating ideas into commercial products to benefit alfalfa and dairy producers. A unit increase in the in-vitro digestibility of Neutral Detergent Fibre (NDF) has been shown to be associated with 0.37 lb/day increase in dry matter intake (DMI) and 0.55 lb/day increase in fat corrected milk yield per cow (Sullivan and Hatfield, 2006). Lignin is an indigestible cell wall component that increases in content with advancing maturity of the alfalfa plant, and is the knocking out a gene coding for a specific lignin biosynthetic enzyme generally results in transgenic plants with significant changes in lignin content and/or lignin composition. The CAI reduced Alfalfa has high protein content, but rapid rumen degradation of alfalfa protein leads to inefficient use. There are two parallel CAI projects designed to improve protein use efficiency: tannin alfalfa and PPO alfalfa. Scientists at the United States Dairy Forage Research Center (USDFRC) predict both approaches will significantly decrease the need for purchased protein supplements and decrease nitrogen losses to the environment on the dairy. Condensed tannins bind with plant proteins, slowing the rate of protein degradation in the rumen. Tannin containing forages, (birdsfoot trefoil) have a slower rate of protein degradation in the rumen, and higher rumen undegradable

protein (i.e. bypass protein). Alfalfa produces condensed tannins, in seed coats, but not in forage tissue. Noble Foundation Scientists are working to modify alfalfa to produce tannins in leaves and stems (Tian, *et al.*, 2008). USDFRC models estimate that alfalfa containing condensed tannins will significantly decrease feeding of protein concentrates and reduce N-losses on dairy farms by 40% (Grabber *et al.*, 2004). Post-harvest protein proteolysis of alfalfa protein during the making of silage often results in >50% Non Protein Nitrogen (NPN) in alfalfa haylage. Red clover, however, has significantly less postharvest proteolysis during ensiling than alfalfa. USDFRC scientists have identified a key enzyme, polyphenol oxidase (PPO), related to this benefit and have expressed this red clover gene in alfalfa. PPO and specific *o*-diphenol substrates combine to form a quinone, which effectively blocks post harvest proteolysis (Sullivan and Hatfield, 2006). Further metabolic modifications are underway to replicate the red clover PPO (Polyphenol Oxidase) system in alfalfa. Plant biotechnology offers exciting new opportunities for crop improvement. In particular we that expect that new traits in alfalfa will greatly improve the crop as a source of fiber and protein for high producing dairy cows fundamentally changing, or re-inventing the crop (Martin, *et al.*, 2005). Animal feeding trials will be needed to validate *in vitro* proof of concept, and to design feeding rations that optimize value of these novel forages for dairy producers. The CAI is an example of the type of interdisciplinary research structure required to design and implement the required research, and to eventually commercialize this exciting technology

3.1 PROTECTION OF PROTEIN, AMINO ACIDS AND FAT

Rumen enzymes degrade the protein to form ammonia and then the ruminal microbes use this non-proteinous nitrogen to synthesize the microbial protein. To increase the efficient utilization of degradable protein, it should be protected from ruminal degradation through chemical

treatments such as formaldehyde and physical treatments like heat treatment and extrusion cooking. Some researchers have reported 33% reduction in protein degradability by formaldehyde treatment of groundnut cake. Fat can be protected using the saponification of fat with calcium salts. Feeding calcium soaps of fatty acids, which are inert in rumen, to negative energy balance animals, enhances dietary energy density and thus, energy intake without compromising the activity of rumen microflora. Thus, the deleterious effect of negative energy balance on animals can be alleviated.

3.2 GENETIC MANIPULATION OF MICROBES

The rumen microbes can be altered genetically to increase their cellulolytic ability and reduction in methanogenesis to improve the overall utilization of feed. This can be done to eliminate the antinutritional factors in feeds and also increase the essential amino acid specially limiting amino acids synthesis by rumen microbes. Attempts are being made to introduce the lignin breakdown property into ruminal microbes. Depolarization of lignin by lignase enzyme which is produced by the soft-rot fungus (*Phanerochaete chrysosporium*) can be useful for the animals (Tien and Kirk, 1983). Efficiency and stability of lignase gene has been modified by Recombinant DNA technology.

3.3 THE RUMEN AND ITS MICRO-ORGANISMS

As the utilization of forages by ruminants depends on microbial fermentative digestion, the principles of digestion in the rumen are discussed as a framework to view the requirements for biotechnology innovations in nutrition.

The rumen is the dominant feature of the digestive tract of ruminants. This maintains a medium that supports a dense and varied population of microorganisms. These organisms ferment feed

materials to produce mainly short chain organic acids (VFAs), methane and carbon dioxide and the process provides substrate (the feed) and ATP (energy) for the growth of micro-organisms.

The microbial mix in the rumen is complex and highly dependent on diet. The main agents that break down fibre, sugars, starches and proteins in the rumen are all anaerobic and include bacteria, protozoa and fungi.

The bacteria are the principal organisms that ferment plant cell-wall carbohydrates (Hungate, 1966) but the anaerobic phycomycetous fungi may at times be extremely important. Protozoa are now recognised as having an overall negative effect in the rumen, particularly where ruminants are fed forage diets low in true-protein (Bird *et al.*, 1990). Protozoa ingest and digest bacteria and reduce the bacterial biomass in the rumen (Coleman, 1975) and consequently the protein supply to the animal. Thus, they decrease the protein to energy ratio in the nutrients absorbed and increase the requirement of animals for true protein. The net result of the presence of protozoa is an increased requirement for dietary bypass protein and on low protein diets a decreased efficiency of utilisation of feed for growth and milk production (Bird *et al.*, 1990).

The presence of protozoa in the rumen may also reduce the rate at which bacteria colonise and degrade the ingested feed particles. In studies with sheep fed straw based diets, it has been found that the apparent digestibility of dry matter was increased by 18% after protozoa had been removed from the rumen (i.e. defaunated) (Bird and Leng, 1984; Soetanto, 1986). This research indicates that large increases in productivity may be achieved with ruminants fed fibrous diets, particularly those low in true protein by controlling or removing protozoa from the rumen. Other workers have not seen the differences in digestibility and in some instances removal of protozoa

from the rumen has led to decreased digestibility of mixed starch containing diets (Jouany and Ushida, 1990).

3.4 FERMENTATIVE EFFICIENCY IN THE RUMEN

A deficiency of a nutrient needed by rumen micro-organisms reduces microbial growth efficiency which reduces microbial biomass and eventually reduces digestibility and feed intake, particularly of fibrous feeds.

The first priority in feeding ruminants is to ensure no deficiencies in the diet of nutrients for microbial growth in the rumen. Of major importance is that the efficiency of microbial growth also determines the proportion of digested feed that is converted to methane and VFA. Methane production accompanies the formation of acetate or butyrate, whereas methane and VFA production are inversely related to microbial cell production.

3.5 MEETING THE REQUIREMENTS FOR EFFICIENT MICROBIAL GROWTH IN THE RUMEN

For crop residues and low-digestibility forages, the primary limitation to the growth of rumen micro-organisms is probably the concentration of ammonia in rumen fluid. The second consideration is deficiencies of minerals, particularly sulphur, phosphorus, magnesium and certain trace minerals. Ammonia in the rumen must be above a critical level for a considerable period of the day to ensure a high rate of microbial growth and digestion and therefore feed intake. The level of ammonia that supports the optimal population of micro-organisms in the rumen the highest protein to energy ration in the nutrients absorbed, and therefore maximum digestion, will vary among diets. In general on forage based diets the ammonia level should be above 200 mg nitrogen/litre (Leng, 1981).

However, that any nutrient, (including many minerals required in the growth of micro-organisms that is deficient in a diet will result in low microbial cell yield relative to VFA and lead to a low protein from microbes to energy (from VFA) in the nutrients absorbed

The ratio of protein digested and absorbed from the intestines to the VFA produced in and absorbed from the rumen is termed the Protein to Energy (P/E) ratio.

3.6 CONSEQUENCES OF THE RUMINANT MODE OF DIGESTION

One of the consequences of the ruminant mode of digestion is that fermentation results in up to 20% of the digestible energy intake being lost as heat and methane. A second major disadvantage is that proteins that are fermented in the rumen are not then sources of amino acids for the animal because they are hydrolysed and their constituent amino acids deaminated by microbes.

In general, where ruminants are fed forage based diets typical of that available in tropical developing countries, small amounts of extra nutrients are needed to balance nutrient availability to requirements. Proteins which are directly available to the animals and are protected from degradation increase the efficiency of anabolism of the absorbed nutrients for growth, pregnancy, lactation or work (Leng, 1981).

3.7 QUANTITATIVE ASPECTS OF FERMENTATIVE DIGESTION IN THE RUMEN

The end products of rumen fermentative digestion are governed by the feed, the rate of consumption of feed, the balance of nutrients in the feed for microbial growth and the balance of micro-organisms that develop in the rumen (bacteria, protozoa and fungi).

In general, a proportion of the digestible feed dry matter is converted to VFA, methane and carbon dioxide and the balance is assimilated into microbial cells. Microbial cells that are

synthesised from the feed resource use the ATP that is generated in the formation of VFA from the feed to provide the energy for synthesis. The microbes are lost from the rumen pool either by passage out of the rumen to be partially digested in the intestine or by death and breakdown within the rumen (with formation of VFA, CO₂ and methane). Lysis and degradation in the rumen is inefficient as it makes the protein of microbes unavailable as such to the animal.

Microbial cells are more reduced than the substrate fermented, as such the quantity of microbial cells leaving the rumen per unit of carbohydrate consumed is related to methane production. The efficiency of microbial growth is then a primary determinate of the quantity of methane produced.

3.8 PROTEIN UTILISATION BY RUMINANTS

Protein that is fermented in the rumen is largely wasted as a source of amino acids to the animal because dietary protein is degraded and essential amino acids are deaminated to form ammonia and VFA. Fermentation of 1 g of protein generates only half the ATP that would be produced from 1 g of carbohydrate and therefore anaerobic microbial growth on protein is approximately half that on carbohydrate. In combination, these effects result in only 30 to 60 g of microbial protein becoming available to the animal for digestion for every kilogram of dietary protein that is fermented in the rumen. The fermentation of protein is, however, associated with relatively small amounts of methane production. On the other hand methane is not generated when protein bypasses the rumen.

Protein that is insoluble, or has a high component of disulphide bonds or is associated with tannins tends to bypass rumen fermentation but is digested in the intestines and in this way it augments the microbial protein and alters the ratio of protein to energy (P/E) in the nutrients absorbed.

CHAPTER FOUR

4.0 ENSURING A BALANCED NUTRITION FOR RUMINANTS ON FORAGE BASED DIETS

The first priority for improving the utilisation of low digestibility forage by ruminants is to optimise the availability of nutrients from fermentative digestion by:—

(i) ensuring that there are no deficiencies of microbial nutrients in the rumen and therefore the microbes in the rumen grow efficiently and, through fermentative activity, extract the maximum possible amounts of nutrients from the forage (i.e. the ratio of microbial cells to VFA produced is high as are production rates of these end products);

(ii) ensuring that the microbial cells (which provide most of the protein to the animal) synthesised in the rumen are not lysed and fermented in the rumen but are available for digestion and absorption as amino acids from the intestines.

The second objective of a feeding strategy should be to optimise the efficiency of partitioning of absorbed nutrients into product by:

supplementation with critical nutrients that escape or bypass rumen fermentation to augment and balance the nutrients absorbed to those required for maintenance of homeostasis, maintenance of body temperature, exercise (or work), and the particular physiological or productive function.

As the nutrients needed for different functions differ in priority, supplementation strategies will need to vary according to climate, environment, management and production targets in any one location.

4.1 MINERAL REQUIREMENTS OF RUMEN MICROBES

The rumen microbes have specific requirements for both macro and micro minerals to meet the needs of structural components of cells and for components of enzymes and co-factors. Little is known about the requirements of the microbial milieu for trace elements and as a ‘rule of thumb’ it is accepted that if the animal is not deficient then it is unlikely that the rumen microbes will be deficient.

Satter and Suttle (1974) have so aptly put the situation, “it will rarely be possible to approach a suspected mineral deficiency situation with a table of minor nutrient requirements or biochemical criteria in the hand, and define a scale of the animal health (microbial health) problems”. In practice, either no mineral supplements are used or a “shot gun” mixture is given as a salt licks (McDowell *et al.* 1984) or as molasses (which is concentrated plant juice rich in minerals) suitably fortified with minerals (Kunju, 1986).

As with any deficiency of a nutrient the likely scenario of a mineral deficiency for rumen organisms is first a reduced growth efficiency of microbes (lowered ratio of cells to VFA produced) with or without a decrease in digestibility. As the deficiencies become more extreme the digestibility of forage must decrease along with the decrease in microbial pool size and it is only then that feed intake will decrease. Feed intake however, will be decreased as P/E ratio decreases if the animal is heat stressed. Correction of deficiency will obviously reverse these effects.

4.2 REQUIREMENTS FOR AMMONIA NITROGEN

Ensuring adequate ammonia N in the rumen to supply the majority of nitrogen for microbial growth is the first priority in optimising fermentative digestion of forage. Satter and Slyter (1974) suggested that 50–80 mg NH₃-N/l of rumen fluid was the optimum for maximising microbial growth and this has been widely accepted. However, recent studies from two laboratories in Australia have clearly indicated that the minimum level of ruminal fluid ammonia for optimum voluntary intake of low N, low digestibility forage by cattle is about 200 mg N/l, even though the digestibility of the forage (in nylon bags) was optimised below 100 mg NH₃-N/l (Krebs and Leng, 1984; Boniface *et al.* 1986; Perdok *et al.* 1988). All these studies were carried out in hot environments and the effects on feed intake are possibly explained by an improving P/E ratio in the nutrients absorbed, which reduces the metabolic heat load.

4.3 TIMING OF UREA SUPPLEMENTS AND THE RATIO OF SUGARS AND STARCHES TO FIBRE IN A DIET

Supplements must provide adequate levels of ammonia in the rumen for continuous growth of both fibrolytic and saccharolytic organisms. The only satisfactory approach to meeting these changing requirements for ammonia is to provide ammonia continuously. One way of doing this is to provide salt/urea or molasses/urea licks and allow the animal to take these as needed. There are indications that cattle and buffaloes given continuous access to multi-nutrient blocks based on molasses/urea are able to control fairly closely their intake of urea. Once buffaloes were accustomed to molasses/urea blocks they adjusted their intake according to the N content of the basal diet (NDDB—unpublished data). Lambs given wheat straw and molasses/urea blocks also had similar abilities and consistently maintained their rumen ammonia levels above 200 mg N/l (Sudana and Leng, 1986).

4.4 REQUIREMENTS FOR AMINO ACIDS/ PEPTIDES BY RUMEN ORGANISMS

There has been considerable controversy concerning the requirements for peptides/amino acids by rumen microbes for efficient growth. A number of studies, however, have reported results of *in vivo* studies which appear to have indicated no apparent requirement for amino acids for efficient growth of rumen organisms (Leng and Nolan, 1984). The results of studies by Maeng *et al.* (1989) showed contradictory results. The studies of these researchers indicated that rumen microbes growing on different carbohydrate substrates have requirements for different N-substrates; cellulolytic organisms may not require amino acids to the same extent as organisms growing on starches or sugar as the major substrate. For microbes utilising sugars and growing on starches there was an apparently high requirement for preformed amino acids/peptides but this was not so for cellulolytic organisms.

Maeng *et al.* (1989) also showed an increase in efficiency of microbial growth on fibrous carbohydrates with decreasing dilution rate of rumen contents. If true this may be advantageous to ruminants given low quality forages that must be retained in the rumen for a considerable period to allow digestibility to be optimised. At the same time the improved ratio of cells: VFA yielded (i.e. P/E ratio) along with the increased availability of the total nutrients are both advantageous. Such a mechanism would advantage an animal with a comparatively slow turnover rate of rumen contents (Devendra, 1989) or fauna-free vs. faunated animal (Bird and Leng, 1985) or animals at high environmental conditions versus cold stressed animals (Young, 1983).

4.5 AMINO ACID REQUIREMENTS OF MICROBES DIGESTING FIBRE

The organisms in the rumen that are largely responsible for the fermentation of cellulose (*Ruminococcus albus*, *Ruminococcus flavefaciens* and *Fibrobacter succinogenes* (previously

called *Bacteroides succinogenes*) appear to have minimal requirements for amino acids and grow on ammonia (Leng, 1981). Conversely, organisms important in starch hydrolysis (*Butyrivibrio fibrisolvens*, *Bacteroides ruminicola*, *Selenomonas ruminantium*, *Streptococcus bovis* and *Ruminobacter (Bacteroides) amylophilus* (Hobson et al. 1988) readily incorporate amino acid N and in many cases peptides (Leng 1981).

Supplementation of sheep fed a poor quality forage with branched chain VFA has been reported to increase the apparent flow of microbial-N to the duodenum. The apparent stimulation of microbial growth with branched chain VFA has also been shown to increase feed intake on occasions (Hemsley and Moir, 1963). This together with the suggested requirements for peptides/amino acids by rumen organisms has tempted many scientists to explain the increased feed intake of ruminants, on poor quality forages that are supplemented with bypass protein, to the slow release of amino acids, peptides and branched chain fatty acids to the rumen milieu from the protected protein (Hunter, 1988; Silva and Ørskov, 1988), even though in most studies there was no evidence of increased digestibility with such supplements in predominately fibre based diets.

The above discussion indicates that the cellulolytic organisms in the rumen even of cattle on straw based diets, are rarely if ever deficient in amino acids, peptides or branched chain VFA in the rumen (Maeng *et al.* 1989). This is not to deny that these organisms may still need these nutrients in “catalytic” amounts but that they are rarely if ever at such low concentrations in rumen fluid to bring about a deficiency.

4.6 THE ROLES OF SMALL AMOUNTS OF FRESH FORAGE IN STRAW BASED DIETS

Farmers in developing countries have generally recognised the benefits to cattle of adding a small amount of fresh green herbage to straw based diets. These practices, which have evolved through trial and error, may have a number of beneficial effects which include the supply of vitamin A, essential minerals, ammonia, peptides/amino acids in an otherwise unsupplemented diet.

Recently it has been shown that where the supplemental forage in a straw based diet given to sheep is of high digestibility a boost to digestibility of the basal diet occurs even at relatively small levels of supplementation (Juul-Nielsen, 1981; Silva and Ørskov, 1988a; Ndlovu and Buchanan-Smith, 1985). The rate of digestibility of straw depends on the rate and extent of colonisation of fibre and the biomass of adherent organisms (Cheng *et al.* 1989) and the high digestibility forage supplement may act to seed microbes onto the less digestible straw. On the other hand, other influences cannot be ruled out. For example, in the studies of Silva and Ørskov (1988) in the absence of an effect of supplemental forage on digestibility, the rumen ammonia levels were often not significantly below 200 mg NH₃-N/. Where increases in digestibility of the basal forage occurred to supplemental forage the ammonia levels in the rumen were significantly below 200 mg N/L and the supplement apparently improved the concentration to above the critical level (Leng 1981). Significantly below 200 mg N/L and the supplement apparently improved the concentration to above the critical level (Leng 1981).

CHAPTER FIVE

5.0 NUTRITIONAL IMPROVEMENTS – VALUE ADDED FEED STUFF

Some of the restrictions which the nutritionist face during feed formulation are the antinutritive factors like trypsin inhibitors, saponins, tannins, phytates, oxalates, high fiber, limitation of phosphorus content etc in grains. Developing genetically customized grains that improves nutritional values might solve these problems.

5.1 LOW PHYTATE CORN

All plant feed ingredients contain natural phosphorus, which is only 30 % accessible, and the rest 70 % is in the form of phytate phosphorus. If grains with low phytate phosphorus and high available phosphorus were made available, the use of supplemental inorganic phosphorus like dicalcium phosphate in poultry would come down (Stillborn and Crum, 1997). This would not only reduce the cost but also high quality, bioavailable phosphorus would be made available to the birds. The added advantage is that less phosphorus would be thrown in the litter and manure, which would lead to the control of eutrophication. This problem is not observed in ruminants because their digestive tract is more efficient in utilizing dietary phosphorus.

Broiler feeding studies with low phytate phosphorus and conventional corn showed that low phytate phosphorus corn was biologically more available based on increased bone ash, bone phosphorus and bone calcium compared to the phosphorus from conventional corn. There was a significant decrease in the fecal phosphorus when low phytate phosphorus corn was used. The low phytate phosphorus corn cultivars contain approximately 35 % phytate phosphorus and 65 % of non-phytate phosphorus, which was reverse in commercial corn (Li *et al.*, 2000).

5.2 HIGH OIL CORN

Study on making of high oil has been going on for a long time. Araba (1997) reported that high oil corn varieties were produced using traditional genetic breeding, which contained 6–8 % oil, but the yield was low compared to commercial varieties. The Dupont company has come out with a brand called most favorable 80 high oil corns through genetic modifications. This variety contains 87 % higher crude oil fat and 3.3% higher crude protein compared to typical corn (Zinn, *et al.*, 1987).

Feeding studies with high oil corn on broilers showed that there was a significant enhancement in body weight and feed conversions (Engelke., 1997, Lee *et al.*, 2001). The broilers fed on high oil corn had less abdominal fat, compared to broilers fed conventional corn and comparable levels of poultry oil. Yan *et al.* (1987) reported that hens fed on high oil corn diet had a better feed to egg ratio. The egg yolks when analysed contained increased levels of linoleic acid and oleic acid.

5.3 LOW OLIGOSACCHARIDE SOYBEAN

Soybeans contain raffinose and stachyose the oligosaccharides, which act as anti nutritive factors (Coon *et al.*, 1990). Oligosaccharides are known to cause osmotic carthisis in laboratory animals. Companies like Dupont have already developed heritably modified soybeans with low oligosaccharides. These cultivars gave an increase of 3% in amino acid digestibility and 5% increase in dry matter digestibility (Leske *et al.*, 1995). Soybeans with high lysine are also being developed to increase the lysine content from 3% to 4.5% and this would reduce the supplemental addition of lysine in diets and the same could be done with corn.

5.4 GENETICALLY MODIFIED CROPS WITH IMPROVED AMINO ACID PROFILES

Genetically Modified (GM) Crops have great potential to decrease nitrogen excretion in poultry. Nitrogen can contaminate ground and surface waters; contribute to acid rains, which increase the acids in soils. Increased levels of essential amino acids like lysine, methionine, tryptophan, threonine in grains would mean that the essential amino acid requirement of poultry can be met with lower protein diets. Feeding these GM varieties would reduce the amount of nitrogen in the form of urea from being excreted into the environment.

The inclusion of GM feedstuffs in animal feed could also pose certain risks. Genetically Modified plants are produced by transferring foreign genes of particular characteristics into feed grain crops. For instance introducing antibiotic resistant marker genes may render common infectious diseases untreatable or certain proteins may cause allergic reactions to animals and humans. Hence proper laboratory, field assessments as well as health assessments have to be made before release of such plants for commercial cultivation.

CHAPTER SIX

6.0 FEED ADDITIVES

6.1 USE OF EXOGENOUS FIBROLYTIC ENZYMES

Enzyme preparations for ruminants are evaluated primarily on the basis of their capacity to degrade plant cell walls and are of bacterial (mostly *Bacillus* spp.) and fungal (mostly *Trichoderma longibrachiatum*, *Aspergillus niger*, *Aspergillus oryzae*) origin (Pendleton, 2000). It is relevant to mention that most of the fibrolytic enzymes used as feed additives in ruminant diets were originally developed as silage additives (Lee *et al.*, 2000). Differences in the relative proportions and activities of individual enzymes have an impact on the efficiency of cell wall degradation. Even within a single microbial species, the types and activity of enzymes produced can vary widely depending on the strain selected and the growth substrate and culture conditions employed for enzyme production. Lee *et al.* (2000) carried out a study on efficacy of an exogenous dose of fibrolytic enzymes (mainly cellulose and xylanase) in cows and found that total digestibility of nutrients was dramatically increased by enzymes. Morgavi *et al.* (2000) found a synergistic effect between ruminal and exogenous fibrolytic enzymes, which increased the hydrolytic potential within the rumen environment. They concluded that synergism is likely a significant mechanism by which enzyme additives improve feed digestion. For increasing fibrolytic activity and increasing post-ingestion quality of forages, Wallace (2001) and Wang *et al.* (2001, 2004) found that by applying the fibrolytic enzymes onto feed prior to feeding was more effective than dosing directly into rumen. Contrary to these results, there are some studies which indicate that the use of exogenous microbial feed enzymes did not result in significant increase in the digestion of fibrous diets in ruminants (Bowman and Sowell, 2003). Finally, there is comparatively more evidence that suggest an improvement in fiber digestion within the rumen

on addition of fibrolytic enzymes either directly into the rumen or pre-treating the feed with the enzymes.

6.2 INCREASING DIGESTIBILITY OF LOW-QUALITY FORAGES

Low-quality forages are a major component of ruminant diets in the tropics. Thus, much progress can be made by improving their quality. The characteristic feature of tropical forages is their slow rate of microbial breakdown in the rumen with the result that much of the nutrients of the feed are voided in the faeces. The slow rate of breakdown also results in reduced outflow rate of feed residues from the rumen which consequently depresses feed intake (Bertin *et al.*, 2005). At present, the main treatment methods for forages such as cereal straws are either mechanical (example: grinding), physical (example: temperature and pressure treatment) or a range of chemical treatments of which sodium hydroxide or ammonia are among the more successful (Wallace *et al.*, 2001). The lignification of the cell walls prevents degradation by cellulase or hemicellulase enzymes. Fortunately, it is possible to use lignase enzyme produced by the soft-rot fungus (*Phanerochaete chrysosporium*) which causes a high degree of depolymerisation of lignin (Wang *et al.*, 2001; 2004). The enzyme acts like a peroxidase and causes cleavage of carbon-carbon bonds. At present the levels of the lignase enzyme produced by the basidiomycete fungi are insufficient for the treatment of straw on a commercial scale. However, it is conceivable that the use of recombinant DNA engineering techniques will allow the modification of the lignase genes and associate proteins to increase their efficiency and stability. The lignin gene has to date been cloned and sequenced from *P. chrysosporium* (Bertin *et al.*, 2005).

6.3 IMPROVING NUTRITIVE VALUE OF CEREALS

According to Morgavi *et al.* (2000) moderate protein content and low amounts of specific amino acids limit the nutritive value of cereals and cereal by-products (barley is low in lysine and threonine). This is a major limitation in the ration formulation for non-ruminant livestock which necessitates addition of expensive protein supplements. There are on-going studies to enhance the low level of lysine in barley by genetically engineering the grain genome. Genetic modification through insertion of genes into rice protoplasts and generation of transformed plants has already been achieved.

6.4 REMOVING ANTI-NUTRITIVE FACTORS FROM FEEDS

Anti-nutritive factors in plant tissues include protease inhibitors, tannins, phytohaemagglutinins and cyanogens in legumes, and glucosinolates, tannins and sinigrin in oilseed rape (*Brassica napus*) and other compounds in feeds belonging to the *Brassica* group. As with amino acid deficiencies, the adverse effects of these compounds are more marked in non-ruminants than in ruminants (Tricarico *et al.*, 2006). Conventional plant breeding has been used to reduce and, in some cases, eliminate such anti-nutritive factors. An example is the introduction of cultivars of oilseed rape which are low in, or free from erucic acid and glucosinolates. 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 as animal feeds. Transgenic rumen microbes could also play a role in the detoxification of plant poisons (Fallon and Earley, 2004b) or inactivation of anti nutritional factors. Successful introduction of a caprine rumen inoculum obtained in Hawaii into the bovine rumen in Australia to detoxify 3-hydroxy 4(1H) pyridine (3, 4 DHP), a breakdown product of the non-protein amino acid mimosine found in *Leucaena* forage (Tricarico *et al.*, 2006; Chevaux and Fabre, 2007) demonstrates the possibilities.

6.5 IMPROVING NUTRITIVE VALUE OF CONSERVED FEED

The conservation of plant material as silage depends upon anaerobic fermentation of sugars in the material which in turn is influenced by the ability of naturally occurring lactic acid bacteria to grow rapidly on the available nutrients under the existing physical environment. Unless the ensiled material is sterilized, lactic acid bacteria are always present. However, the ensiling conditions may not always be ideal for their development (Alshaikh *et al.*, 2002; Lila *et al.*, 2004). In addition to the number and type of bacteria, other interrelated factors may affect quality of silage, including availability of water-soluble carbohydrates, the dry-matter content, the pH and extent of air exclusion. For example, lack of water-soluble carbohydrates may be overcome by wilting the material to raise the dry matter to a level at which less acid is required to stabilize the fermentation. The availability of sugars in the material and the rate at which the different micro-organisms multiply also influences the ensilage process. Throughout this century, research workers have investigated ways through which the fermentation process in silage making can be controlled in order to improve the feeding quality of the resulting silage. Use of additives, to restrict the activity of the microorganisms, to stimulate the fermentation by the lactic acid bacteria or simply as nutrients has been one of the approaches (Alshaikh *et al.*, 2002; Chevaux and Fabre, 2007). Additives used in the early studies included chloroform, toluene and cresol (to inhibit bacterial growth) and sulphuric acid and hydrochloric acid (to reduce the pH). Indeed, over the last 40 to 50 years, corrosive, acid-containing additives have been widely used in silage making (OJEU, 2004a). Other fermentation inhibitors which have been studied include organic acids, salts of acids, formaldehyde and other aldehydes, sodium hydroxide, and antibiotics. Of these, formic acid is probably the most widely studied and has been reported to have a beneficial effect on the fermentation process and on the nutritive value of silage. Sulphuric acid is cheaper than formic acid and is common in some countries. However, acids are

a hazard on the farm and can be particularly dangerous if recommended to uninformed farmers. Salts of acids are safer to handle but are less effective than the acids from which they are derived. The hazardous nature of some of the chemical additives has necessitated a search for alternative compounds for improving the ensilage process. A group of compounds classified as fermentation stimulants have been widely studied. These include sugar sources (molasses and whey), enzymes and inocula of lactic acid bacteria. Molasses is of particular relevance to smallholder farmers in developing countries in the tropics where sugar-cane is produced and processed. 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, *Aspergillus Niger* or *Trichoderma viridi* (Spruzs and Selegovska, 2004) increases the amount of available sugars. Commercial hemicellulase and cellulase enzyme cocktails are now available and improve the fermentation process considerably (Fallon and Earley, 2004b). However, prices of these products preclude their viability for farm level application, especially in developing countries. There are two forms of indigenous lactic acid bacteria: the homofermentative type which converts hexose sugars to lactic acid with no loss of dry matter and the heterofermentative type which produces a range of compounds accompanied by loss of dry matter as carbon dioxide. Thus, the native bacteria are not the most efficient. Considerable research in the USA and Europe has been directed towards the development of microbial silage additives (inoculants) (OJEU, 2004b). Commercial bacterial inoculants designed to add sufficient homofermentative lactic acid bacteria to dominate the fermentation are now available. The objective of using such additives is to ensure the rapid production of the required amount of lactic acid from the carbohydrates present to preserve the ensiled material. Most of

such inoculants contain acidophilus, *Pediococcus acidilactive* and *Streptococcus thermophilus* (Sara *et al.*, 2004). In general, the results with bacterial inoculants have been quite variable. However, with an effective product, it is possible to improve the fermentation of low dry-matter silages and to enhance the efficiency of their utilization. In order to improve the effectiveness of microbial inoculants in breaking down structural carbohydrates to glucose, detailed knowledge of the lactobacilli bacteria is essential. Work already undertaken on the molecular biology of *Lactobacillus plantarum* and other species (Agovino, 2006) suggest that the rapid progress in this area will make it possible to construct novel genes encoding highly active fibre-degrading enzymes. Such genes could then be inserted into strains of *L. plantarum*. Successful silage making incorporating these technologies can only be achieved with strict adherence to recommended application procedures, including rates of additives, inoculants. This technology is available in most developing regions including Africa. However, it is not fully exploited. Indeed, in Africa silage making is still generally restricted to large-scale commercial farms (Spruzs and Selegovska, 2004). Suphalucksana and Soyong (2006) reported on the ascospores of *Chaetomium cupreum* AN102 growing in rice straw and para-grass, the main feed for ruminants, may possible to develop as biological animal feed for ruminant. The study revealed that sterilized rice straw and para-grass were rapidly degraded by applying the specific strain of *C. cupreum* for one month. The feed composition analyses revealed that *Chaetomium* treated with those organic substances gave the highest potential to degrade cellulose and followed by hemicellulose and crude fibre, respectively, which significantly different when compared to the non-treated one. Moreover, *Chaetomium* treated in sterilized para-grass gave significantly higher nitrogen free extract (NFE) than non-sterilized para-grass which indicated that *C. cupreum* AN102 could be used to improve the ruminant nutrition through biodegradation.

6.6 IMPROVING RUMEN FUNCTION

Sara *et al.* (2004) have reviewed the major areas of rumen function which might benefit from transgenic technology. These include development of transgenic bacteria with enhanced cellulotic activity, capability to cleave lignohemicellulose complexes, reduced methane production capability decreased proteolytic and/or deaminase activities, increased capability for nitrogen "fixation" and increased ability for microbial production of specific amino acids. The first successful transfer of foreign genes into rumen bacteria (*Bacteriodes ruminicola*) was reported by (Fallon and Earley, 2004a, 2004b). However, we are still a long way from commercial production of genetically engineered rumen bacteria. Although several workers have isolated genes encoding plant structural carbohydrate-degrading enzymes from rumen bacteria, there are limited reports (Bertin *et al.*, 2005) on the genetic engineering of these microorganisms. In contrast to conditions in which single species of organisms are grown in controlled environments and where the energy supply is usually in excess of demand, the rumen environment is very complex, competition between different microbial species is intense and energy is usually the limiting growth factor (Fallon and Earley, 2004a, 2004b). This is probably the main reason why reintroduction of genetically modified rumen bacteria into their natural habitat has met with variable success. Advances being made in transformation methods for obligate anaerobic bacteria will certainly result in successful genetic engineering of a range of rumen bacteria. However, it is not possible to predict if any of these bacteria will be capable of colonizing the rumen. It can 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. Modification of rumen microbial population (Sinclair *et al.*, 2006) is one such opportunity. However, technical difficulties associated with making genetic modifications to individual species of rumen bacteria (Sinclair *et al.*, 2006) hinder progress in

this area. One of the most common explanations for improved animal health when ruminants are fed a Direct Fed Microbials (DFM) suggests that beneficial microbes compete with potential pathogens and prevents their establishment. Direct Fed Microbials may also produce antimicrobial end products such as acids that limit the growth of pathogens (Denev, 2006). Additionally, metabolism of toxic compounds and production of stimulatory substances has resulted from feeding DFM to ruminants. In contrast, there are many bacterial based DFM that are sold for use in ruminant diets with more specific applications. These products often contain *Lactobacillus* spp. being one of the most common microorganisms used. Other commonly used bacteria include various species of *Bifidobacterium*, *Enterococcus*, and *Bacillus* (Denev, 2006). Most bacterial-based DFM are probably beneficial because they have effects in the gut and not in the rumen (Denev, 2006). For example, *L. acidophilus* produces lactic acid, which may lower the pH in small intestines to levels that inhibit the growth of pathogenic microbes (Denev, 2006). Early research with DFM in ruminants first involved applications for young calves fed milk, calves being weaned, or cattle being shipped (Zheng *et al.*, 2000). These animals were thought to be stressed and have immature microbial ecosystems in their guts (Denev *et al.*, 2000). Cattle that are shipped are often provided limited feed and water for prolonged periods of time during transit and may have abnormal environments in their guts that could lead to establishment of pathogenic microbes. Large doses of beneficial microorganisms have been hypothesized to recolonize a stressed intestinal environment and return gut function to normal more quickly in scouring calves. However, the data supporting such claims have been questionable. Calves fed *L. acidophilus* had reduced incidence of diarrhea (Fallon and Earley, 2004a) and reduced counts of intestinal coliform bacteria (Kamra *et al.*, 2002). However, feeding bacterial DFM to calves had no beneficial effects in other studies (Zheng *et al.*, 2000). The beneficial use of lactic acid-

producing bacteria (largely *L. acidophilus*) in young calves and as fermentation stimulants in ruminants (Nursoy and Baytok, 2003) have been described by many other investigators. However, the beneficial effects of these bacterial supplements have not been clearly defined. Supplementation of lactobacilli may be useful in the close-up dry period of lactation when intake is depressed and animals are stressed. A field trial was conducted on a commercial dairy to study the effects of feeding a DFM consisting of 2 strains of the specific bacteria *Enterococcus faecium* plus yeast on prepartum and postpartum performance of 366 Holstein cows. Cows received either the DFM or a placebo from about 10 days before expected calving until 23 or more days after calving (Nursoy and Baytok, 2003). Supplementation with DFM (Direct Fed Microbials) increased milk fat percentage in the first-lactation cows, increased milk protein percentage in the second and greater lactation cows, and decreased the number of antibiotic treatments given to the second-lactation cows (Oetzel *et al.*, 2007).

6.7 EFFECTS ON MICROBIAL POPULATION AND RUMINAL FUNCTIONS

Yeast cultures (YC) are very beneficial in the rumen. Several reasons for improvements in ruminal fermentation from feeding YC have been suggested. Numerous studies (Jouany, 2001; Alshaikh *et al.*, 2002; Lila *et al.*, 2004; Tricarico *et al.*, 2006; Chevaux and Fabre, 2007) documented positive effects of yeast Culture not only on the rumen environment, but also on the improvement of microbial activities. Yeast cultures have caused beneficial changes in activity and numbers of rumen microbes. Yeast supplements stimulate the growth of beneficial microorganisms in the rumen. For example, the numbers of total ruminal anaerobes (Nocek *et al.*, 2003; Jouany, 2001) and cellulolytic bacteria (Dann *et al.*, 2000; Jouany, 2001) have been increased with YC. According to Alshaikh *et al.* (2002) yeast culture obviously improve the cellulolytic activities of rumen microorganisms in such a way that they increase their total

numbers, improve fiber digestion, reduce lactate accumulation, reduce the concentration of oxygen in rumen fluid and improve utilization of starch supplied in the feeding ration. In this way they influence (inhibit) the rate of VFA production and, thus, increase the stability of rumen environment and improve the intensity of digestion. Yeast cultures have also directly stimulated rumen fungi, which may improve fiber digestion (Jouany, 2001). They increased the number of rumen protozoa and neutral detergent fiber digestion in steers fed straw-based diets (Nikkhah *et al.*, 2004). Yeast cultures have also been shown to stimulate acetogenic bacteria in the presence of methanogens (Jouany, 2001), which might result in a more efficient ruminal fermentation. Jouany (2001) and Strohlein (2003) stated that some yeast strains showed a better capability to use lactate because they stimulated its utilization by propionic acid bacteria. The utilization of lactate by these bacteria is of the major importance for the stabilization of rumen environment. Bachs (2006) and Chevaux and Fabre (2007) observed that in individual animals yeasts reduced daily fluctuations in pH values and also decreased differences existing between them. This resulted in a higher stability of rumen environment during the day. Increased metabolism of lactic acid should theoretically raise ruminal pH and this may be one reason why these YC increased numbers of rumen cellulolytic bacteria and improved fiber digestion (Dann *et al.*, 2000). Nikkhah *et al.* (2004) reported that *S. cerevisiae* was able to prevent the accumulation of lactic acid production by competing with *Streptococcus bovis* for glucose and by stimulating the uptake of lactic acid by *Megasphaera elsdenii* perhaps by supplying amino acids and vitamins. In contrast, added yeasts was unable to prevent acute episodes of lactic acidosis when fermentations were challenged with a diet rich in fermentable carbohydrates (Nocek *et al.* 2003; Jouany, 2001).

Adding specific nutrients to feed improves animal digestion and thereby reduces feed costs. A lot of feed additives are being currently used and new concepts are continuously developed.

6.8 ADDITION OF VACCINES AND ANTIBODIES TO FEEDS

Many feeds and fodder are from crop plants that have been modified for characteristics such as disease or pest resistance and their nutritive value remain unaffected. Secondly, plants are used as bioreactors for the production of recombinant biopharmaceuticals like cytokines, hormones, monoclonal antibodies, bulk enzymes and vaccines (Miele, 1997)

6.9 PREBIOTICS AND PROBIOTICS

Probiotics are live microbial feed supplements which beneficially affect the host animal by improving the intestinal microbial balance (Madan, 2003). The most common probiotics are lactic acid producing bacteria.

Microorganisms are naturally present in the digestive system of the animals. Some microbes aid digestion, others can potentially cause pathogenesis. The implication of gut ecology for nutrition, feed conversion and disease control, the microbial ecology of the gut merits greater attention. Use of antibiotics disturbs the microbiological balance of gut flora eliminating most of the beneficial flora. On stopping the antibiotic treatment, pathogens begin to reestablish themselves in the intestine. Overgrowth of these organisms and subsequent invasion of the system by pathogenic organisms cause inflammatory, immunological, neurological and endocrinological problems. Using probiotics can help build up the beneficial bacteria in the intestine and competitively exclude the pathogenic bacteria. These bacteria also release enzymes, which help

in the digestion of feed. The concept of using probiotics or direct fed microbials in animal feed particularly poultry and aquaculture is slowly becoming popular. The common organisms in probiotic products are *Aspergillus oryzae*, *Lactobacillus acidophilus*, *L. bulgaricus*, *L. plantarium*, *Bifidobacterium bifidium*, *Streptococcus lactis* and *Saccharomyces cerevisiae*. These products can be administered through water or incorporated in the feed. Probiotics have been particularly useful in the early stages of chick growth since the gut of the newly hatched chick is sterile and administering probiotics through water at this stage helps to build up beneficial bacteria much faster than the normal course.

The most important quality parameter of probiotics is that the vegetative or the spore forms have to be viable to be able to multiply in the gut. Secondly they should be resistant to antibiotics, which are administered so that the gut ecology could be maintained (Rowland *et al.*, 2009). Genetic engineering would help develop probiotics with special properties like secreting enzymes and vitamins in large quantities. Such products would be the future generation feed additives.

Probiotics have also been used in a big way as pond cleaners in aquaculture. Probiotic bacteria directly uptake or decompose the organic matter or toxic material and improve the quality of water. The microbial cultures produce a variety of enzymes like amylase, protease, lipase, xylanase and cellulase in high concentrations than the native bacteria, which help in degrading waste. These bacteria have a wide range of tolerance for salinity, temperature, pH which usually exist in aquaculture operations (FAO/WHO, 2001). The use of antibiotics in aquaculture is banned due to rejection of export consignments of marine products. Hence usage of probiotics is propagated to counter the effect of viral and bacterial infections in commercial aquaculture. The

pond probiotics also have a special blend of denitrifying bacteria that remove the algae's primary source of food and nitrogen from the water. This drastic reduction in nitrogen concentration makes it difficult for the algae to bloom. The balance between phytoplankton, zooplankton and beneficial bacteria during culture period play a crucial role in the maintenance of pond health. There is yet no definitive parameter to judge the efficacy of probiotics (Penna *et al.*, 2008). A quick and easy microbiological testing kit would be very useful in evaluating pond health on a daily basis.

In cattle there is no supplemental probiotic given since the ruminant animals employ microbial fermentation to digest the food. By the use of genetic engineering one could alter rumen microorganisms and populations to provide more efficient feed conversion, improved milk composition and removal of toxins. The usage of probiotics has been a subject of intense research all over the world and has been accepted as an alternative for antibiotics.

The concept of prebiotics in feed is fairly recent. Prebiotics are basically feed for probiotics where they are resistant to attack by endogenous enzymes and hence reach the site for proliferation of gut microflora. Some of the prebiotics which are currently used in animal feed are Mannan-oligosaccharides MOS (Mannan Oligosaccharide base Nutritional Supplement) fructo-oligosaccharide and mixed oligo-dextran. Mannan-oligosaccharides are mainly obtained (Mannan Oligosaccharide from cell walls of yeasts. Other sources of MOS are copra or palm kernel meal. MOS interferes with the colonization of the pathogens. Cell surface carbohydrates are primarily responsible for cell recognition. Bacteria have lectins (glycoprotein) on the cell surface that recognize specific sugars and allow the cell to attach to that sugar. Binding of *Salmonella*, *E.coli* and *Vibrio* sp. is shown to be mediated by a mannose specific lectin like

substance present on the bacterial cell surface. Similarly fructo-oligosaccharides from chicory have been used as prebiotics to competitively exclude pathogenic bacteria (Xu *et al.*, 2003). The pH of the lumen gets reduced thus preventing the entry of pathogenic bacteria. The concept of using prebiotics has not yet been accepted but the advantages of prebiotics are that it can stand high pelletizing temperatures in the feed and also have a long shelf life.

6.10 DIETARY AMINO ACIDS

Essential amino acids are added as supplement to the feed to get a balanced amino acid profile. Since the amino acid profiles of the ingredients do not match the profile of amino acid requirement of the specific species, supplemental essential amino acids are added. The new trend is to formulate diets on digestible amino acid levels thereby reducing the requirement of protein. Lysine and methionine have been used as supplements. Lysine is produced by microbial fermentation and methionine is chemically synthesized. Genetically enhanced micro-organisms are being used to produce threonine and tryptophan on a commercial basis and soon other essential amino acids also would become available. Using all these amino acids it is possible to lower dietary crude protein level by 2 – 3 %, which is a substantial saving for the farmer. The -
*concept of an ideal protein blend from GM feedstuffs and feed additives (such as amino acids and enzymes) will greatly help with decreasing the amount of nitrogen excreted in animal waste.

6.11 TOXIN BINDERS

Feed manufacturers have been incorporating different form of inhibitors in their diets to prevent mycotoxin formations. A variety of substantial, chemical and biological approaches to counteract the mycotoxin problem have been reported, but large scale, realistic and cost effective means for detoxification of mycotoxins containing feed stuffs are limited Most of them are fungistats and

not fungicides meaning they only inhibit growth of molds and do not inactivate any toxins already present. Present day methods are generally use of organic acids and their salts like propionic acid or adsorbents like bentonites, zeolites and hydroxyl aluminosilicates. In the future, biotechnology based products like microbes, herbal extracts or esterified glucomannan could be used. Aqua extracts of garlic, onion, turmeric, neem have been shown to exert antifungal activity or inhibit aflatoxin production. Identification of the active ingredients would help in the development of genetically modified herbs with enhanced activities to make them cost effective.

6.12 MINERALS AND VITAMINS

The absorption and availability of inorganic trace minerals varies depending upon the nature of the minerals (sulphate, oxide or carbonate), their solubility, ionization. Trace minerals are now being attached to oligopeptides to make them more bioavailable. Commercial preparations of proteinated selenium and chromium are used in poultry. In the case of vitamins due to varying availability and stability of vitamins in ingredients supplemental vitamins are incorporated in diets. These vitamins are much more stable than naturally occurring forms. Stability is achieved through the application of advanced technologies which involve preparation of biologically active derivatives, coating technologies, carriers and diluents (Mertens, 2002).

6.13 BYPASS PROTEIN

The national dairy development board has introduced certain new concepts in feeding and nutrition of cattle. The production and distribution of quality cattle feed is a critical requirement for enhanced production. Bypass protein is a protein alternative and National the Dairy development board (NDDB) has produced urea molasses mineral block (UMMB). UMMB

(Urea Molasses Mineral Block) has fermentable nitrogen, energy and minerals. Ruminants can synthesize protein from non-protein nitrogen like urea and energy is obtained from molasses. This product is very useful in green fodder deficient areas. Such concentrates can also be made in the future if bagasse can be commercially used after delignification.

6.14 METABOLIC MODIFIERS

Metabolic modifiers like recombinant bovine somatotropin (rBST) have been used to increase efficiency of production such as weight gain or milk yield per feed unit), improve carcass composition (meat-fat ratio). In developed countries like USA, its use has led to an increase of 10-15% of milk yield. Similarly, researchers have also developed porcine somatotropin that increases muscle growth and reduces body-fat deposition, resulting in pigs that are leaner and of greater market value

Metabolic modifiers are a group of compounds that modify animal metabolism in specific and directed ways. They have the overall effect of improving productive efficiency (weight gain or milk yield per feed unit) improving carcass composition (lean:fat ratio) (Milligan L.E., (1971) in growing animals, increasing milk yield in lactating animals and decreasing animal waste per production unit (NRC 1994) Two classes of compounds have received major focus, one the somatotropins (STS) and the other adrenergic agonists. Both the metabolic modifiers are already in use in the United States and are also approved by several countries. Commercially these compounds are produced by the use of recombinant DNA technology to selectively produce specific components for a species.

The most common somatotropin is the bovine somatotropin (bST) which is administered to dairy cows. This leads to increased milk yields, improved productive efficiency (milk/feed) and

decreased animal waste. Similarly somatotropins have also been used in pigs which resulted in greater nutrient use. Administering of β adrenergic agonist components lead to improved feed conversion ratio, daily weight gain and carcass leanness (NRC 1994). These components induce changes in endocrine and cellular mechanism

CHAPTER SEVEN

7.0 BIOTECHNOLOGY AND MONOGASTRIC NUTRITION

Most developing countries are experiencing difficulties providing sufficient food (staples) for their resident population. In general in most third world countries food production is increasing at a slower rate than the population growth rate. Developing countries on average are increasing food imports by 10% per year and this is likely to continue into the foreseeable future. Whilst ruminants need not compete with humans for food there are few economic, large scale systems for pig and poultry production established in developing countries which do not rely on grain based concentrate or on crops such as potatoes and other root crops which also take up land that could potentially be used for human food production. The recent developments of sugar cane as a source of feed (juice) for pigs, however, has a major advantage which resides in the high biomass production per hectare and a great potential to form the basis of an integrated farming system (Preston, 1990).

There has been far too little emphasis on developing systems of production of pigs and poultry on agro-industrial by-products, the exception being the molasses and swill-feeding systems for pigs that have developed in Cuba (Figueroa, 1989).

By-products high in sugar such as molasses, palm oil sludge and fruit and vegetable pulps lend themselves to feeding to monogastrics. The constraints to production are a low protein content and in some case an associated high level of fibre which is an intestinally indigestible carbohydrate.

Pigs are able to utilise some fibrous feed, possibly up to 30% of their digestible feed intake because of caecal fermentation. It appears desirable that in the future, pig production should utilise at least a proportion of fibrous carbohydrate polymers. Free-range pig production, where

breeding stock obtain a fair proportion of their feed from pastures is a good beginning forced on producers in Europe by the high cost of grain feeding in intensive fattening systems.

If the microbial ecosystem in the caecum of the pig is well developed, basal diets high in sugar and low in fibre combine well with vegetable proteins such as cottonseed meal which are fibrous.

7.1 POTENTIAL AREAS FOR BIOTECHNOLOGY IN PIG NUTRITION

The use of oil-seed protein residues for pigs and poultry are generally limited by the fibrous components and in some cases the presence of secondary plant compounds which may be toxic or simply lower production (e.g. gossypol in cottonseed meal). To overcome the constraint created by too much fibre it may be possible to develop pre-treatment to protect the protein and then find mechanisms for decreasing the fibre (e.g. growing fungi, such as the white rot species on the materials to decrease fibre and increase protein) or find methods to hydrolyse the cellulose to simple sugars before feeding.

Chemical treatment of the feed to hydrolyze fibre to monosaccharides may also be potentially possible (e.g. with pressurised steam and sulphur dioxide).

Delignification with say *Coprinus* fungi and treatment by pressure/steam of fibrous crop residues to produce simple sugars for inclusion in pig rations is a further possibility requiring research.

At least one group in the U.K. is attempting to introduce cellulase genes from *Clostridial* sources into embryos of pigs with the concept of producing pigs with a capacity to digest more cellulose. The enzyme, if expressed will be controlled by a promoter sequence encoding only in the pancreas (this is given in a little more detail below as an example of (a) the possibilities, (b) the problems and (c) the requirements for basic research prior to any developments which might be applied (Hazelwood Gilbert, 1989).

7.2 NON-CEREAL FEED RESOURCES FOR PIGS

The most promising alternatives to cereal grains for intensive feeding of pigs in the tropics are: sugar cane juice (Mena, 1983; Sarria *et al.*, 1990; Speedy *et al.*, 1991), sugar cane molasses (High-test, “A” or “B” molasses) (Figueroa and Ly, 1990), juice from the sugar palm tree (FAO/TCP, 1994a), oil, whole fruit and by-products of the African oil palm (Devendra, 1992; Ocampo *et al.*, 1990; Ocampo, 1992, 1994), cassava roots (Buitrago, 1990) and by-products (Espinal and Ospina, 1993, unpublished data) and organic waste from urban households, restaurants and canteens (Dominguez, 1990; 1991).

Other products and by-products from tree, root and tuber crops, are included in tropical pig diets but mostly on an *ad hoc* basis, and not as the basis of the feeding system.

7.3 PROTEIN SUPPLEMENTATION OF TROPICAL ENERGY-RICH FEED RESOURCES

With the exception of the organic waste of urban origin, all the feed resources mentioned above are characterized by very low levels of protein in the dry matter ranging from 1% in sugar cane juice and molasses to 5% in whole African palm fruit (Alexander, A.G. 1988). One consequence of this low level of protein in the basal diet is that almost all the requirements for amino acids have to be supplied in a supplement. While this could be considered to be a disadvantage, in fact the contrary may be the case because:

- It is much easier, and may be cheaper, to find a supplement already balanced with respect to the essential amino acids (lysine, methionine, etc.) than to make a supplement that will compensate for the imbalanced amino acid profile present in cereal grains.

- The total amount of protein to be offered is less because when it is derived almost wholly from supplements, rather than partially from cereal grains, it is better balanced in the essential amino acids.

These possibilities can be visualized more easily in the comparisons presented, which show that the essential amino acid requirements of sows can be contained in 30–35% less total protein, than what is recommended (Batterham *et al.*, 1993) which is inflated by the presence of excessive amounts of the non-essential amino acids present in cereal grains.

The final point is the relationship between the amount (and therefore cost) of the supplement and the level of animal performance. This is especially important in most tropical countries where the ratio - price of protein: price of energy - is much wider than is the case in temperate (cereal growing) countries. Thus the point on the response curve (to protein) where profit per fattening pig is optimized will not necessarily coincide with the point where biological performance is maximized.

7.4 BIOTECHNOLOGY DERIVATIVES FOR POULTRY FEED

Research in Cuba twenty years ago showed that raw sugar could replace the cereal grain in diets for all classes of poultry (Perez *et al.*, 1968). However, the technology never became truly commercial. Raw sugar is almost always too expensive to use in animal feeds. Molasses and cane juice are economically competitive with cereals but there are many factors that mitigate against their use for fattening and laying birds other than water fowl. For example:

- Large scale poultry systems are designed to use complete mixed and dry diets.

- The productive life of broilers is too short to permit them to adapt adequately to liquid diets.
- The mouth parts of birds are not designed for consuming liquid feeds. There is considerable wastage (whether it is cane juice or molasses) and the feed sticks to the plumage which is an inducement for cannibalism.

7.5 LAYING AND FATTENING HENS

Use of the cane juice as a substitute for grain in broiler and laying hen diets has not been successful due mainly to the physical difficulties experienced by chickens in consuming a low-density liquid diet, and the stress caused by splashing of the sugar-rich juice on the feathers which can lead to cannibalism. Rates of growth and feed conversion have rarely exceeded 60–70% of genetic potential (Arango *et al.*, 1994).

Laying hens, particularly the heavier dual purpose strains, which have been raised on cane juice, have been maintained through complete laying cycles with satisfactory, although lower, egg production (about 65% laying rate) than would be expected with cereal diets (Vargas, J., unpublished data).

7.6 MOLASSES

An interesting development has been reported from Cuba (Rodriguez *et al.*, 1994). It was found that ground sun-dried tropical forages, especially the leaves of sugar cane, were able to absorb up to twice their weight of “B” molasses. The molasses is first diluted with 20% of its weight of water, and then mixed with the dry leaf meal and the mixture left to dry in the sun for 48 hr. The final product contains (DM basis): 70% “B” molasses and 30% dried sugar cane leaf meal. It is friable and easily mixed with other dry ingredients. Its true metabolizable energy value was

found to be 2.87 Mcal/kg DM. Up to 40% of this feed has been included in diets of laying hens with no loss of performance. The aim now is to replace the whole of the cereal grain with this alternative tropical feed resource.

7.7 DUCKS AND GEESE

Recent developments in the feeding of cane juice to ducks are much more promising (Bui and Vuong, 1992; Becerra *et al.*, 1994). Ducks are well adapted to consuming liquid diets and, provided they have access to water for swimming, have no problems with the sugar juice falling on their plumage. It appears to be possible to reach at least 80–90% of genetic potential for growth

CHAPTER EIGHT

8.0 POSSIBILITY OF MODIFYING THE DIGESTIVE FUNCTION THROUGH DEVELOPMENT OF TRANSGENIC ANIMALS

By virtue of their hindgut fermentation, pigs have about the same capacity as humans for digesting plant structural polysaccharides (Engelhardt *et al.*, 1985).

In theory, substantially more energy would be available to pigs (and other simple animals) if cellulose and hemicellulose could be digested in the small intestine and the sugars absorbed. In addition the complex carbohydrates of grains (e.g. β -glucans of barley), fibrous protein sources (e.g. cotton seed meal) could be more efficiently used. In the latter case it would allow a much higher proportion of protein from such sources to be incorporated into the diets replacing more expensive less fibrous protein meals such as fish or meat meal.

The ability to digest cellulose and hemicellulose by intestinal enzymes is not present in any animal. However, if encoding a gene for microbial cellulase or hemicellulase could be incorporated into an animal's genome and expressed in the pancreas it may allow these enzymes to be produced and secreted along with the other pancreatic secretions. This is being tested by Hazelwood and Gilbert (1989) by introducing into the early embryo of mice (and they suggest they will do this with pigs) the cel E gene of *Clostridium thermocellum* which encodes a thermostable endoglucanase with xylan-hydrolysing activity.

Expressions of this gene in transgenic mice will be regulated by the elastase I promoter/enhancer region (from rats) which is located “upstream” of the elastase gene and is responsible for restricting the synthesis of the digestive enzyme elastase to target sites of expression of other genes, to the exocrine enhancer cells in the pancreas of transgenic mice.

To modify porcine digestive enzyme secretion to include the enzymes, cellulase and hemicellulase, will require tissue specific expression of the cel E gene and secretion of mature active endogluconase enzyme into the intestinal lumen. For this reason gene constructs incorporating the elastase promoter/enhancer and cel E coding sequence have been designed.

The concept of transgenic pigs is at the ideas stage, the research will undoubtedly require a return to basic investigations, undoubtedly over and under expression of the genes when transferred will be a major problem.

Fibre breakdown in the pig by cellulase is likely to be slow and therefore gut capacity is likely to be a major constraint even when transgenic pigs are produced.

8.1 PORCINE GROWTH HORMONE (PST)

Undoubtedly porcine growth hormone injected in controlled amounts into pigs on grain based concentrates increase their growth rate, efficiency of growth and reduces fat deposition. The place of PSt in pig production, as it pertains to developing countries, is unknown. The small production units, the likely high cost of injections and the “scavenger system” generally operated at the small farmer level in developing countries suggests that PST is unlikely to be used. The exception may be in grain-based feeding systems for pigs aimed at the markets provided by the higher income groups and tourists.

8.2 TRANSGENIC PIGS — PORCINE GROWTH HORMONE

The technology for production of transgenic pigs has certainly been developed. Problems of over and under expression of the genes are still apparent. Over expression results in considerable problems of leg weakness and reproductive inefficiencies whereas under expression gives no additional benefits. The nutrition of pigs expressing for growth hormone again has been understudied with the usual emphasis being placed on the thrust to produce transgenic animals. The need to develop special feeding and management systems for pigs seems to have been ignored.

Where these techniques have been applied in developed laboratories the absence of disease is mandatory. It is conceivable that in addition to a “special type of nutrition” there may be special requirements for disease and parasite control. Disease and parasitism are more likely to impact heavily on pigs and other animals with a greater potential for growth.

The problems to be faced in pigs transgenic for porcine growth hormone are well summed up in a paper by Wilmut *et al.* (1988):

“The pig exhibiting human growth hormone did not grow any faster than normal pigs but they had less back fat which might be of potential value to pig farmers. Unfortunately, these transgenic pigs also suffered from a number of abnormalities, including arthritis, lack of coordination of their rear legs, susceptibility to stress, anoestrus in gilts and lack of libido in boars. So it is back to the bench to attempt to redesign the gene to avoid these problems.”

CHAPTER NINE

9.0 CONCLUSIONS AND RECOMMENDATIONS

In this review, recent advances in the application of biotechnology to animal nutrition have occurred in three main areas: value-addition to food crops used as animal feed, production of feed additives and the manipulation of rumen microbes to enhance feed utilization. As nutrition appears to represent the most severe constraint to animal production, developing countries can take advantage of the benefits of modern biotechnology and successfully apply it to improve livestock feeding. This is especially important as the world is facing daunting challenges in the face of rapid population growth and climate change. It is therefore important for developing countries to adopt and promote biotechnologies relevant to animal nutrition in the following priority areas:

- Development of appropriate supplements that balance nutrition of ruminants fed poor quality forages, crop residues or agro-industrial byproducts. This requirement includes:
 - development of appropriate supplements of mineral/non protein-nitrogen/other microbial factors, and identification of protein resources naturally protected from rumen degradation
 - processing of protein resources to protect them from rumen degradation
- Development of systems to produce protected protein resources, especially in areas where protein is scarce. These include
 - proteins from crops, trees or industrial by-products
 - aquatic plants algae and animals.
- Chemical treatment (feed additives)

- Microbiological treatment (probiotics)
- Improving fermentation in the rumen using
 - modification of the microbial ecosystem
 - modified microorganisms

Animal production in most developing countries could be increased many fold by finding ways and means of applying these concepts in biotechnology to improve animal nutrition.

Based on this review, the following recommendations can be made:

- Developing countries should place emphasis on local research to manipulate through feeding technology the microbial ecosystem of the rumen and the animals' metabolism to make ruminants as efficient as possible on the feeds that are locally available. When the direct and indirect effects are taken into account this could result in large improvements in productivity which could meet increasing food demand by humans and allow animal numbers to increase.
- The most important milestone will be the production of packaged supplements to complement the available feed resources at the site of dense livestock populations.
- Modern biotechnology should be developed but only in a few selected research institutes and it should be funded from national resources. The areas of research should be carefully chosen to have a local emphasis that is not being researched elsewhere.
- The farmers should be encouraged to produce crops like low phytate corn, high oil corn and low oligosaccharide soybean on a large scale for the production and feeding of animals.

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