Status Papers on Future Research in Sheep Production and Product Development

Climate Change, Green House Gas Emission and Sheep Production

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Climate change is seen as a major threat to the survival of many species, ecosystems and the sustainability of livestock production systems in many parts of the world. Green house gases (GHG) are released in the atmosphere both by natural sources and anthropogenic (human related) activities. An attempt has been made in this article to understand the contribution of ruminant livestock to climate change and to identify the mitigation strategies for reducing enteric methane emission in livestock. The GHG emissions from the agriculture sector account for about 25.5% of total global radiative forcing and over 60% of anthropogenic sources. Animal husbandry accounts for 18% of GHG emissions that cause global warming. Reducing the increase of GHG emissions from agriculture, especially livestock production should therefore be a top priority, because it could curb warming fairly rapidly. Methane (CH_4) with the global warming potential of 25 and longer residence time is an important GHG (Wuebbles and Hayhoe, 2002; Forster et al., 2007). The rising concentration of CH_4 is strongly correlated with increasing populations, and currently about 70% of its production arises from anthropogenic sources (Moss et al., 2000; IPCC, 2007). Domestic ruminants contribute the major proportion of total agricultural emission of methane. Although the reduction in GHG emissions from livestock industries is on high priorities, strategies for reducing emissions should not reduce the economic viability of enterprises if they are to find industrial acceptability.

Ruminants have been recognized as a major contributor to GHG (Steinfeld et al., 2006). Livestock account for mainly 80% of all emissions from the agricultural sector. Emissions into the air by any animal production system can be problematic in terms of pollutants and toxicity and in terms of odour and the perception of air quality by human neighbours. The three major GHGs are carbon dioxide (CO_2), methane and nitrous oxide (N_2O). CH_4 also has serious impact on high atmosphere ozone formation. It is important to reduce methane production from the rumen, because methanogenesis corresponds to 2-12% of dietary energy loss as well as contributing to global warming. Enteric CH_4 emissions represent an economic loss to the farmer where feed is converted to CH_4 rather than to product output.

The livestock production is an integral part of mixed farming systems practiced in the entire length and breadth of the country. The potential impacts of climate change on livestock sector in India are:

i. The anticipated rise in temperature between 2.3 and 4.8°C over the entire country together with increased precipitation resulting from climate change is likely to aggravate the heat stress on sheep, adversely affecting their productive and reproductive performance.

ii. Given the vulnerability of India to rise in sea level, the impact of increased intensity of extreme events on the livestock would be large and devastating for the low-income rural masses.

iii. The predicted negative impact of climate change on Indian agriculture adversely affecting livestock production by aggravating the feed and fodder shortage.

Sheep production system in India is changing rapidly due to change in climate conditions, feed scarcity, urbanization and change in transhumance /migration of sheep rearing. Impact of climate change in a region needs to

be assessed by ambient temperature, relative humidity (RH), difference in maximum and minimum temperature in a day, rainfall, wind velocity, solar radiation, infra-red (IR) radiation, evaporation rate and level of atmospheric CO_2. Heat stress and feed scarcity are the two most important determinants that are likely to alter the sheep production in arid / semi-arid tropical regions. A major proportion of sheep population (>70%) thrives well in arid and semi-arid areas of western region and southern plateau (southern peninsular region). As per the livestock census (GOI, 2003), only five states of the country i.e. Andhra Pradesh, Rajasthan, Karnataka, Tamil Nadu and Jammu and Kashmir cover about 60% of the sheep population. It is clearly indicated that sheep is densely populated in hot-arid and semi-arid regions. The productive potential of sheep in these areas is influenced by the exposure to harsh climatic factors namely, high ambient temperature, solar radiation and wind velocity along with long distance walking in search of feed resources. Drivers of changing climates may force to shift from existing extensive system of sheep management to intensive or semi-intensive management. Given the importance of climate change affecting livestock productivity, this review collates and synthesizes literature on effect of changing climate on sheep production in Indian scenario and the mitigation strategies that need to be addressed to counter such environmental extremes.

While measures to reduce the growth of GHG emissions are an important response to the threat of climate change, adaptation to climate change will also form a necessary part of the response. In this context, adaptation refers to strategies that act to reduce the adverse impacts of climate change. Developing adaptation strategies is therefore an important part of ensuring that countries are well prepared to deal with any negative impacts that may occur as a result of climate change. Given limited resources, adaptation strategies must target those populations most vulnerable to global change and equip those unable to adaptgenerally the poorestwith the tools and incentives that will enable them to do so. Adaptation to climate variability has been an ongoing necessity for the agricultural sector. Existing strategies to manage climate variability present opportunities for meeting the challenges of future climate change.

Sources of green house gases (GHGs)

CH_4 is emitted from a variety of anthropogenic and natural sources (Wuebbles and Hayhoe, 2002; Rotz et al., 2010). Anthropogenic sources include fossil fuel production and use, animal husbandry (enteric fermentation in livestock and manure management), paddy/rice cultivation, biomass burning, and waste management (Kumaraswamy et al., 2000; Mosier et al., 2004). More than 70% of global CH_4 emissions are related to anthropogenic activities (IPCC, 2007) and the remaining from natural sources include wetlands, gas hydrates, permafrost, termites, oceans, freshwater bodies, non-wetland soils, volcanoes and wildfires (Breas et al., 2001). Emissions from enteric fermentation of the domestic livestock contribute significantly to GHGs inventories (Garcia-Apaza et al., 2008). Emissions from animal facilities primarily consist of animal respiration and enteric fermentation. In addition, emissions from manure storage are also believed to be a potential source of CH_4 (Chianese et al., 2009).

Livestock and climate change

There are two sources of GHG emissions from livestock:

i. *Digestive process*: Methane is produced in herbivores as a by-product of 'enteric fermentation,' a digestive process of enzymatic degradation elaborated by symbiotic microbes inhabiting in rumen medium in which carbohydrates are broken down into simple molecules for absorption into the bloodstream (Mc Michael et al., 2007).

ii. *Animal wastes*: Animal wastes contain organic compounds such as carbohydrates and proteins. During the decomposition of livestock wastes under moist, oxygen free (anaerobic) environments, the anaerobic bacteria transform the carbon skeleton to methane. Animal wastes also contain nitrogen in the form of

various complex compounds. The microbial processes of nitrification and de-nitrification of animal waste forms nitrous oxide, which is emitted to the atmosphere (Swamy and Bhattacharya, 2006; Scheehle and Kruger, 2006).

The major global warming potential (GWP) of livestock production worldwide comes from the natural life processes of the animals. Methane production appears to be a major issue although it presently contributes only 18% of the overall warming. It is accumulating at a faster rate and is apparently responsible for a small proportion of the depletion of the protective ozone layer. CH_4 arises largely from natural anaerobic ecosystems, rice/paddy field and fermentative digestion in ruminant animal. In fact, CH_4 is considered to be the largest potential contributor to the global warming phenomenon (Johnson et al., 2002; Steinfeld et al., 2006). It is an important component of GHG in the atmosphere, and is associated with animal husbandry (Leng, 1993; Moss et al., 2000). Much of the global GHG emissions currently arise from enteric fermentation and manure from grazing animals and traditional small-scale mixed farming in developing countries. The development of management strategies to mitigate CH_4 emissions from ruminant livestock is possible and desirable. Not only can the enhanced utilization of dietary 'C' improve energy utilization and feed efficiency hence animal productivity, but a decrease in CH_4 emissions and also reduce the contribution of ruminant livestock to the global CH_4 inventory.

Significance of Indian livestock to GHG emissions

In India, although the emission rate per animal is much lower than the developed countries, instead of huge livestock population, the total annual CH_4 emission is about 9-10 Tg from enteric fermentation and animal wastes (Sirohi and Michaelowa, 2007). India possesses the largest livestock population in the world and accounts for the largest number of cattle (world share 16.1%), buffaloes (57.9%), second largest number of goats (16.7%) and third highest number of sheep (5.7%) in the world (FAOSTAT). Of the various livestock enterprises, dairying is most popular in the country and dairy animals, which comprise of the majority of the livestock, account for nearly 60% of these enteric emissions (Table 1). The GHG emissions from the agriculture sector in India are mainly in the form of CH_4 primarily due to enteric fermentation and rice/paddy cultivation (MOA, 2005). N_2O is also emitted from this sector and is mainly from the agricultural fields due to application of fertilizers. Table 1 describes the contribution of different livestock species to methane pool in India.

Table 1. Contribution of Indian livestock population to methane pool

Species	Population (millions)	Emission/animal (g/yr)	Population contribution (Tg/yr)	Contribution (%)
Cattle	177.84	28616	5.09	54.72
Buffalo	98.70	28616	2.82	30.37
Goat	125.46	7154	0.90	9.70
Sheep	64.27	7154	0.50	5.38

Enteric methane emission

Livestock are produced throughout the world and are an important agricultural product in virtually every country. CH_4 is emitted as a by-product of the normal livestock digestive process, in which microbes resident in the animal's digestive system ferment the feed consumed by the animal. This fermentation process, also known as enteric fermentation, produces CH_4 as a by-product. The CH_4 is then eructated or exhaled by the animal. Within livestock, ruminants (cattle, buffalo, sheep, and goats) are the primary source of emissions. Other livestock (swine

and horses) are of lesser importance for nearly all countries. The number of animals and the type and amount of feed consumed are the primary drivers affecting emissions. Consequently, improvements in management practices and changes in demand for livestock products (mainly meat and dairy products) will affect future CH_4 emissions.

Among the livestock, cattle population contributes most towards enteric CH_4 production (Johnson and Johnson, 1995). Enteric fermentation emissions for cattle are estimated by multiplying the emission factor for each species by the relevant cattle populations. The emissions factors are an estimate of the amount of CH_4 produced (kg) per animal and are based on animal and feed characteristics data, average energy requirement of the animal, the average feed intake to satisfy the energy requirements and the quality of the feed consumed (Sejian and Naqvi, 2011a). The district or county level emission from enteric fermentation is computed as a product of the livestock population under each category and its emission coefficient (Chhabra et al., 2009). The emission coefficients for CH_4 emissions from enteric fermentation are country-specific and these coefficients should conform to IPCC guidelines (IPCC, 2007).

Enteric fermentation-process description: Enteric fermentation is the digestive process in herbivores animals by which carbohydrates are broken down by micro-organisms into simple molecules for absorption into the bloodstream. CH_4 is produced as a waste product of this fermentation process. CH_4 production through enteric fermentation is of concern worldwide for its contribution to the accumulation of GHGs in the atmosphere, as well as its waste of fed energy for the animal. CH_4 is produced in the rumen and hindgut of animals by a group of *Archaea* known collectively as methanogens, which belong to the phylum *Euryarcheota*. Among livestock, CH_4 production is greatest in ruminants, as methanogens are able to produce CH_4 freely through the normal process of feed digestion. Ruminant animals are the principal source of emissions because they produce the most CH_4 per unit of feed consumed. What makes ruminant animals unique is their "fore-stomach" or rumen, a large, muscular organ. The rumen is characterized as a large fermentation vat where approximately 200 species and strains of micro organisms are present. The microbes ferment the plant material consumed by the animal through a process known as enteric fermentation. The products of this fermentation provide the animal with the nutrients it needs to survive, enabling ruminant animals to subsist on coarse plant material. CH_4 is produced as a byproduct of the fermentation and is expelled. "Monogastric" animals produce small amounts of CH_4 as the result of incidental fermentation that takes place during the digestion process. "Non-ruminant herbivores" produce CH_4 at a rate that is between monogastric and ruminant animals. Although these animals do not have a rumen, significant fermentation takes place in the large intestine, allowing significant digestion and use of plant material.

Methane producing bacteria reside in the reticulum, rumen and large intestine of ruminant livestock. These methanogens bacteria, use a range of substrates produced during the primary stages of fermentation to produce CH_4, thus creating generated energy required for their growth. All methanogen species can utilize hydrogen ions (H^+) to reduce CO_2 in the production of CH_4 as this reaction is thermodynamically favorable to the organisms. Availability of H^+ in the rumen is determined by the proportion of end products resulting from fermentation of the ingested feed. Processes that yield propionate and cell dry matter act as net proton-using reactions, whereas a reaction that yields acetate results in a net proton increase. Other substrates available to methanogens include formate, acetate, methanol, methylamines, dimethyl sulfide and some alcohols; however, only formate has been documented as an alternative CH_4 precursor in the rumen.

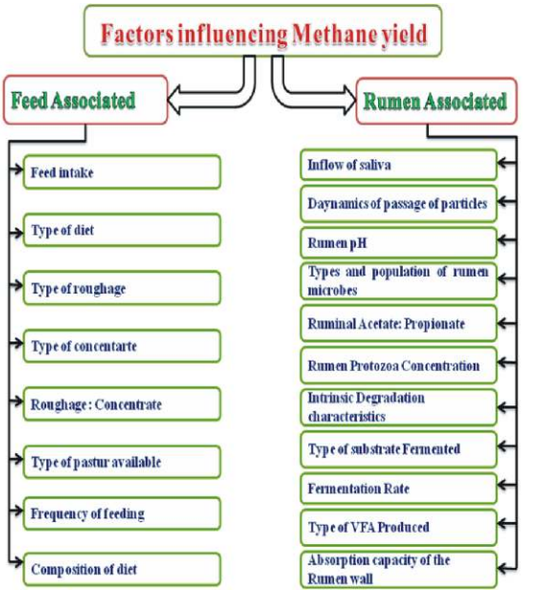
The principal methanogens in the bovine rumen utilize hydrogen and carbon dioxide, but there is a group of methanogens of the genus *Methanosarcina* that grow slowly on H_2 and CO_2 and therefore maintain a distinct niche by utilizing methanol and methylamines to produce CH_4. Formate, which is formed in the production of acetate, can also

be used as a substrate for methanogenesis, although it is often converted quickly to H_2 and CO_2 instead. Volatile fatty acids (VFA) are not commonly used as substrates for methanogenesis as their conversion into H_2 and CO_2 is a lengthy process, which is inhibited by rumen turnover. Therefore, methanogenesis often uses the c and CO_2 produced by carbohydrate fermentation, as VFAs are formed. By removing H_2 from the ruminal environment as a terminal step of carbohydrate fermentation, methanogens allow the microorganisms involved in fermentation to function optimally and support the complete oxidation of substrates. The fermentation of carbohydrates results in the production of H_2 and if this end product is not removed, it can inhibit metabolism of rumen microorganisms.

Factors influencing enteric methane production

The factors affecting enteric methane production are summarized in Plate 1. CH_4 production differs with microbial species and livestock breed. Thus, breeding of livestock on the basis of CH_4 production in an effort to reduce enteric CH_4 emissions without compromising animal productivity is also possible (Hegarty et al., 2007). Rumen pH plays a major role in determining what population of microbes predominates, which also influences the CH_4 concentration (Erfle et al., 1982**)**. Reduction of ruminal pH can decrease ruminal CH_4, which can improve feed utilization in the ruminant animals (Lana et al., 1998). The ratio of ruminal acetate: propionate *in vivo* is highly influenced by the capacity of the bacteria to produce CH_4 *in vitro*. Cattle with low acetate: propionate ratios also have low ruminal pH values, and *in vitro* experiments corroborate the concept that pH has a major impact on CH_4 production and acetate: propionate ratio (Lana et al., 1998).

Methanogens living on and within rumen ciliate protozoa may be responsible for up to 37% of the rumen CH_4 emissions (Hegarty et al., 2007). In the absence of protozoa, rumen CH_4 emissions are reduced by an average of 13%, but this varies with diet. Decreased CH_4 emissions from the protozoa-free rumen may be a consequence of reduced ruminal dry matter digestion, decreased methanogen population, altered pattern of volatile fatty acid production, decreased hydrogen availability, and increased partial pressure of oxygen in the rumen (Yoon and Stern, 1995). Decline in methanogenesis associated with removal of protozoa is strong on high concentrate diets, because protozoa are relatively more important sources of hydrogen on starch diets, and many starch-fermenting bacteria do not produce hydrogen. Because protozoa also decrease the supply of protein available to the host animal, their elimination offers benefits in both decreasing GHG emissions and potentially increasing feed conversion ratio in livestock production (Hegarty, 1999).



The composition of diet fed to the livestock is another important factor which influences CH_4 emission (McCrabb et al., 1997), especially from enteric fermentation in lactating dairy cows. The proportion of forage in livestock diet and the source of grain influence enteric CH_4 production by ruminants (Beauchemin and McGinn, 2005). The amount of digestible nutrients especially the carbohydrate fraction is used to estimate CH_4 emission from livestock with high precision. Furthermore, a diet rich in fat reduces CH_4 formation in the rumen (Jentsch et al., 2007). Puchala et al. (2005) identified the potential of condensed tannins in forages to reduce CH_4 emission by ruminants. The level of CH_4 emission is positively correlated with live weight, dry matter intake (DMI), milk yield (MY) and feeding

level (Yan et al., 2006). To enhance productivity, manipulation of dietary composition of cattle feed is a nutritional management strategy with potential to reduce CH_4 production by dairy cows (Yan et al., 2006).

There are several feed supplements which alter significantly the level of CH_4 emission (Leng, 1991; Moss et al., 1995). But additional feed supplement should be used with caution as they can reduce animal productivity when the wrong dosage is used (Beauchemin and McGinn, 2006; Foley et al., 2009). Incremental inclusion of malic acid in beef cattle diets results in linear reductions of both the total daily emission of CH_4 and its emissions expressed per unit of DMI. However, the dietary inclusion of malate is also associated with a decline in DMI (Martin and Streeter, 1995; Martin et al., 2008). This could potentially decrease animal performance, with consequent increases in lifetime CH_4 emission owing to extended age to slaughter. Therefore, further *in vivo* research is required to clarify the long term effects of malate supplementation on CH_4 emission, animal performance and productivity (Foley et al., 2009). Beauchemin and McGinn (2006) demonstrated that canola oil (*Brassica campestris L*) can be used to reduce CH_4 emissions from cattle, but animal performance may be compromised due to lower feed intake and decreased fiber digestibility. Essential oils have no effect on CH_4 emissions, whereas fumaric acid causes potentially beneficial changes in ruminal fermentation but no measurable reductions in its emissions. Certain fats and oils are potential natural CH_4 reducing feed compounds, and are effective even at common dietary proportions (Machmuller et al., 1998). McGinn et al. (2004) demonstrated that sunflower (*Helianthus annuus L*) oil, ionophores and possibly some yeast products can be used to decrease the gross energy lost as CH_4 from cattle, but fiber digestibility is impaired with oil supplementation.

Research involving *in vitro* and *in vivo* experiments indicates that high grain feeding reduces CH_4 emission in farm animals (Lana et al., 1998; Hristov et al., 2001). There are two possible reasons for this high grain-fed reduction in CH_4 emission viz., declined methanogenesis as well as lower acetate: propionate ratio (Christophersen et al., 2008). Not only can enhanced utilization of dietary C improve feed utilization efficiency and animal productivity, but a decrease in CH_4 emissions can reduce the contribution of ruminant livestock to the global CH_4 inventory (Johnson and Johnson, 1995). Best management practices (BMPs) can significantly reduces the emission of CH_4 per unit of animal weight gain. Management-intensive grazing (MIG) is a BMP that offers the potential for more efficient utilization of grazed forage crops via controlled rotational grazing and more efficient conversion of forage into meat and milk **(**DeRamus et al., 2003). There are several factors which play a major role in influencing enteric fermentation and as a result these factors play a significant role in controlling the overall CH_4 emission from these livestock species. There is an urgent need to understand these factors affecting variability in enteric CH_4 production to decrease the uncertainty in GHG emission inventories and to identify viable reduction strategies (Beauchemin and McGinn, 2005).

Modeling of green house gases

In the wake of the current global climate crisis, it has become increasingly clear that there is an urgent need to not only better understand the magnitude of the livestock sector's overall contribution to GHG emissions, but also to identify effective approaches to reduce emissions. Enteric fermentation, manure management and farmland activities are the major sources of GHGs from farms. Since on-site measurement of GHG emissions from livestock production facilities requires complex and often expensive equipment, estimates of emissions from individual farms or from different farming systems may need to be made by means of prediction equations. Some models have been developed specifically to predict GHG emissions from animals (Ellis et al., 2007) and others have either been modified or adapted to estimate GHG emission from the entire farm (Chianese et al., 2009; Rotz et al., 2009). Computer simulation provides a cost - effective and an efficient method of estimating GHG emissions from dairy farms and data inputs from management scenarios may affect generated GHG emissions results (Chianese et al., 2009). The

Integrated farm system model (IFSM) has been used effectively in dairy farms to predict the whole farm GHG emissions. IFSM predicts the effect of management scenarios on farm performance, profitability and environmental pollution (Rotz et al., 2009). Broadly, there are two types of models present, viz., empirical/statistical and mechanistic/dynamic Model. At present, among two models, mostly mechanistic models are used to estimate CH_4 emissions from enteric fermentation at a national and global level.

Significance of modeling: Agricultural production is recognized as a significant contributor to GHG production (Amon et al., 2006; Monteny et al., 2006). Intensive dairy production in particular, contributes significant quantities of CH_4 and several forms of nitrogen which can contribute to nitrous oxide production (Casey et al., 2005). Over the past ten years, research studies have attempted to address various sources of GHG emissions within the dairy production system. These sources have included housing (Amon et al., 2001; Ellis et al., 2001), manure removal, storage and treatment systems (Amon et al., 2006; Berg et al., 2006). Others have compared GHG emissions from conventional farming practices to those employed in organic production (Olesen et al., 2006; Weiske et al., 2006). Many of these studies have looked at one section of the production chain in isolation. But dairy production is a complex system involving inputs such as feed and fertilizer, animals with inherent physiological structures for fermentation of feedstuffs and the production of manure, storage systems, cropping systems and export of meat and milk.

It is very easy to understand that attempting to design and conduct research trials to ascertain the effect of one or multiple changes on production, economics and GHG emissions from a dairy production system would be expensive and time consuming. Therefore, the use of whole farm models, with short-term studies for validation, is an attractive alternative. In simulating the whole farm production process, IFSM allows for the evaluation and comparison of alternative agronomic, feeding, manure storage and disposal strategies in terms of production, profitability and nutrient cycling. The model also accounts for the use of fossil fuels used in the production process. The model does not predict production of GHG but does provide the basic information required to predict GHG (CH_4 and nitrous oxide) emissions using factors published in the scientific literature.

Types of models: Modeling is an integral part of the scientific method in nutritional research. A mathematical equation or model can be viewed as an idea, hypothesis or relation expressed in mathematics. Models of rumen function, aim at an improved prediction of fermentation in the rumen for practical purposes (e.g., microbial representations in protein evaluation systems) or at an improved understanding and integration for research purposes. Such quantitative approaches may be broadly classified into empirical and mechanistic models. CH_4 production is generally predicted on the basis of equations involving DMI, intake of carbohydrates, digestibility and intake of dietary energy, animal size, milk components and digestibility of dietary components. The Intergovernmental Panel on Climate Change (IPCC) publishes guidelines for national GHG inventories (IPCC, 1997) that are used for official estimates of CH_4 emissions. The models developed for prediction of CH_4 emission can be classified into two principal groups: (1) empirical (statistical) models that relate nutrient intake to CH_4 output directly, and (2) dynamic mechanistic models that attempt to simulate CH4 emissions based on a mathematical description of ruminal fermentation biochemistry (Sejian et al., 2011a). Empirical models use experimental data to quantify relationships directly. In contrast, mechanistic models are constructed by examining the structure of a system and analyzing the behaviour of the system in terms of its individual components and their interactions.

Empirical models: Methane (CH_4) production by ruminants through enteric fermentation has been predicted using simple equations since the 1930s and 1940s (Kriss, 1930; Bratzler and Forbes, 1940). All empirical models either work on dry matter intake or the combination of milk yield and body weight to explain CH_4 yield. Additional variables in the models that are related to CH_4 yield are digestibility, carbohydrate type and proportion of roughage in dietary dry matter. The are several empirical models based on total dry matter intake (Kriss, 1930; Axelsson, 1949), digestible

carbohydrates (Bratzler and Forbes, 1940; Moe and Tyrell, 1979), digestibility and feeding level (Blaxter and Clapperton, 1965), milk yield and live weight (Kirchgessner et al., 1991), dry matter intake and feed characteristics (Yates et al., 2000) and a range of animal and dietary factors (Holter and Young, 1992; Yan et al., 2000; Mills et al., 2003). CH_4 production from these databases was estimated from nonlinear equations relating Y (CH_4 energy as a percentage of metabolizable energy) to gross energy intake and dietary grain level. Schils et al. (2005) calculated CH_4, N_2O and CO_2 emissions with refined emission factors. The CH_4 emission from enteric fermentation is calculated with emission factors per kg DM uptake, distinguishing between concentrates, grass products and maize silage.

Tier 1 and 2 methods are two methods of estimating CH_4 emissions from enteric fermentation given by IPCC in its revised reference manual (IPCC, 1997). The Tier 1 method is a simplified approach that relies on default emission factors (EF) drawn from previous studies. Therefore, only readily available animal population data are needed to estimate emissions. The Tier 2 method is a more complex approach that requires country-specific information on livestock characteristics and manure management practices. In Tier 2, average daily feed intake (in terms of energy content, MJ d^{1}) and CH_4 conversion rates are used to estimate CH_4 emissions. For example, feed intake in dairy cows is estimated from body weight, average daily weight gain, feeding situation, average daily milk production, and average amount of work performed per day, percentage of cows that give birth in a year and feed digestibility.

Empirical models have been developed to predict CH_4 emissions from manure storages and the majority of empirical manure storage models are biologically based. The model of Chen and Hashimoto (1980), applied to fresh manure, was primarily developed to simulate anaerobic digesters, and several of the parameters were empirically determined based on data from digesters. Zeeman (1994) used emission factors to predict the actual emission of CH_4 from digested manure. The model of Sommer et al. (2004) simulates the production and emission of CH_4 from manure storages based on the degradation of volatile solids (VS).

Mechanistic models: Mechanistic models are important tools for assessing mitigation options and for directing experimental research towards options most likely to result in significant reduction of CH_4 emissions from enteric fermentation. The impact of mitigation strategies to reduce CH_4 emissions has to be assessed holistically, and empirical models lack the biological basis for such an assessment. Various mechanistic models have been developed that account for the most important features of ruminal digestion and microbial metabolism. The majority of dynamic mechanistic models appearing in the nutritional literature are based on systems of ordinary differential equations. Several mechanistic models of rumen function contain a hydrogen gas balance sub-model from which CH_4 can be predicted. Central to CH_4 prediction in these mechanistic models is the accurate prediction of hydrogen production from fermentation of substrates to volatile fatty acids (VFA) and subsequent hydrogen utilization for various purposes. The representation of VFA stoichiometry is likely to have the largest impact on CH_4 prediction.

In contrast to the empirical models, these models take into account the on sequences of feed intake level on the effective concentrations of substrates and different classes of micro-organisms that co-exist in the rumen (Bannink and Dijkstra, 2006), both of which are determinant factors for the extent of substrate degradation. Several dynamic, mechanistic models that estimate CH_4 emissions have been developed (Baldwin et al., 1987; Mills et al., 2001). MOLLY (Baldwin, 1995 and its current version MOLLY, 2007) developed at the University of California, is a dynamic mechanistic model of nutrient utilization in cattle. Ruminal CH_4 production was predicted based on hydrogen balance. Excess hydrogen produced during fermentation of carbohydrates and protein to lipogenic VFA (acetate and butyrate) is partitioned between use for microbial growth, bio-hydrogenation of unsaturated fatty acids, and production of glucogenic VFA (propionate and valerate). The VFA stoichiometry in MOLLY is based on equations

developed by Murphy et al. (1982). The rumen model of Dijkstra et al. (1992) based on a series of dynamic, deterministic and nonlinear differential equations and only it considers the interaction among several classes of micro-organisms enabling the representation of intra-rumen recycling of microbial matter. CH_4 production in the rumen and hindgut was introduced by Mills et al. (2001) following the principles of Baldwin (1995). Mills et al. (2003) used forage proportion of the diet in addition to DMI to predict CH_4 production. The model describes the fate of excess hydrogen (H_2) produced during fermentation, from the production of lipogenic volatile fatty acids (VFA) and microbial growth on amino acids. Later, Kebreab et al. (2004) incorporated the rumen model to a whole animal model that simulates CH_4, N and P output from ruminants given various types of diets. Bannink et al. (2006) developed a new stoichiometry for fermentation within the rumen based entirely on experimental observations with lactating dairy cows; therefore, COWPOLL was modified to accommodate these stoichiometric coefficients. One of the fundamental differences in estimating CH_4 emissions between MOLLY and COWPOLL is the representation of microbes in the rumen and the coefficients of fermentation for transformation of substrate to VFA. Mills et al. (2001) further refined the model of Dijkstra et al. (1992) and included new estimates of VFA yield which Bannink et al. (2006) had derived from *in vivo* data. Recently, these VFA yields were re-estimated and made dependent on rumen acidity (Bannink and Dijkstra, 2006). The effect of acidity of rumen fluid was added as an explanatory variable in regression studies of in vivo data on rumen fermentation in lactating cows (Bannink et al., 2005a). The resulting new values were applied by Bannink et al. (2005a, b) in an evaluation of CH_4 emission on several farms in practice and by Dijkstra et al. (2006) in evaluating the development of CH_4 emission by dairy cows. Ellis et al. (2007) formulated the most accurate equations which could be useful to the livestock industry for accurately predicting CH_4 production from a minimum set of inputs.

Whole farm modeling: Livestock is recognized as an important emitter of GHGs, but little quantitative data exist on emission rates and the effect of management on these emissions. Simple process-level relationships were integrated in the development of a comprehensive model for predicting all important sinks and emission sources to determine a whole-farm carbon balance and an estimate of the net farm emission of greenhouse gas. Relationships were used to track carbon dioxide, CH_4, and nitrous oxide flows during crop production, from the animals and from manure on the barn floor, during storage and following land application. These relationships were added to the Integrated Farm System Model to predict net greenhouse gas emissions along with nitrogen and phosphorus losses and the overall performance and economics of farm production systems.

The development of whole-farm approaches for the mitigation of GHG emissions has been taken up recently by several research groups. A common feature of whole farm models is the ability to calculate CH_4 and N_2O emissions from dairy farms. Furthermore, the models vary considerably on many other aspects. General characteristics of whole farm models include: model type, CH_4 and N_2O emissions, CO_2 emissions, C sequestration, NH_3 and NO_3 emissions, P cycling, pre-chain emissions, animal welfare, economics, biodiversity, product quality, soil quality and landscape aesthetics. Whole Farm Model (WFM) uses pasture growth and cow metabolism for predicting CH_4 emissions in dairy farms. Also included in the WFM is climate and management information. Some WFMs are developed by Neil et al. (1997), Sherlock et al. (1997) and Bright et al. (2000). However, these models are adequate only for predicting CH_4 production by non-lactating Holstein cows. Prediction rates for lactating cows are less accurate and WFMs currently described in the literature seem inappropriate (IPCC, 1997). Hence, developments of WFMs are required for the prediction of nutrient and GHG emissions and better estimates of enteric CH_4 production. Currently available WFMs may incorrectly estimate CH_4 emission levels because they cannot predict the wide range in enteric CH_4 emissions as affected by DMI and diet. The low prediction accuracy of CH_4 equations in current WFMs may introduce substantial error into inventories of GHG (Sejian et al., 2011).

Integrated Farm System Model (IFSM) is a simulation model that integrates the major biological and physical processes of a crop, beef, or dairy farm and evaluating the overall impact of management strategies used to reduce CH_4 emissions. IFSM is a process based, whole farm simulation including major components for soil processes, crop growth, tillage, planting and harvest operations, feed storage, feeding, herd production, manure storage, and economics (Rotz et al., 2009). IFSM predicts the effect of management scenarios on farm performance, profitability, and environmental pollutants such as nitrate leaching, ammonia volatilization, and phosphorus runoff loss. The Dairy Greenhouse Gas Model (Dairy GHG) is a type of IFSM which was developed to provide an easy to use software tool for estimating greenhouse gas emissions and the carbon footprint of dairy production systems.

Techniques for estimation of enteric methane

There is a growing interest in changing the management strategies to reduce enteric CH_4 production without negatively influencing animal productivity. This theme has been the focus of much research to improve agriculture's environmental sustainability. However, accurate CH_4 measurements are required for identifying mitigation strategies that can discriminate among treatments relevant to on-farm conditions (Lassey, 2008). Several techniques are used to quantify enteric CH_4 emissions including whole animal chambers (Grainger et al., 2007), and sulfur hexafluoride (SF_6) tracer technique (Pinares-Patiño et al., 2008; McGinn et al., 2009). Estimates on GHG emission from animal feeding and waste management are based on country specific emission factors (Yang et al., 2003) and when the local data are unavailable, the default emission factors recommended by IPCC (1997) guidelines are used.

Measurement of CH_4 emissions from individual animals has traditionally been made with open-circuit respiration chambers, which are highly accurate and reliable for animals offered indoor diets. However, these chambers are not as suitable for evaluating emissions for grazing animals. A new technique that makes use of an inert gas (SF_6) has recently been developed for determining CH_4 emissions from cattle and sheep under grazing conditions. The SF_6 tracer technique enables the determination of enteric CH_4 emissions from both individual as well as on a large number of animals (Vlaming et al., 2007). This technique is based on the use of a controlled release bolus containing SF_6 gas, which is inserted into the animal's rumen. DeRamus et al. (2003) described the apparatus and collection methods of the SF_6 tracer method. The technique slowly samples the mixed eructated and respired air from the animal, generally over a 24 h period. This air sample is then analyzed for the ratio of CH_4:SF_6 concentration and the ratio is multiplied by the known release rate of SF_6 emitted from a permeation tube placed in the rumen (Pinares-Patiño et al., 2008). The technique is extremely useful to examine grazing management influences on enteric CH_4 emissions (Pinares-Patiño et al., 2007). In addition they concluded that the SF_6 tracer technique can be used with a reasonable degree of accuracy for inventory purposes and for evaluating the effects of mitigation strategies on CH_4 emissions.

A more flexible technique for quantifying emissions is to model the dispersion of a target gas from the source (Flesch et al., 2004), so that a downwind concentration of gas can establish the emission rate. This "inverse-dispersion" technique has the advantage of simplicity, as it requires only a single gas concentration measurement and basic wind information (McGinn et al., 2006). However several important factors must be considered to get accurate results from this technique. These factors are: (1) ambient condition including landscape with clearly defined wind regime as well as not having other nearby emission sources, (2) duration of measurement with long line-average concentration measurements, (3) period specific measurements and ignoring periods known to be problematic for inverse-dispersion calculations, and (4) lactation specific concentration measurement that allows one to ignore wind complexity in some lactations.

A micrometeorological mass difference technique is used to measure CH_4 production by cattle in pasture and feedlot conditions (Harper et al., 1999**)**. Measurements are made continuously under field conditions, semi-automatically for several days. The method permits a relatively large number of cattle to be sampled. These techniques do not infringe on the measurement being made, and are generally nonintrusive (Laubach and Kelliher, 2004). Limitations include light winds, rapid wind direction changes, and high-precision CH_4 gas concentration measurement. The mass difference method provides a useful tool for "undisturbed" measurements on the influence of feedstuffs and nutritional management practices on CH_4 production from animals and for developing improved management practice for enhanced environmental quality (Harper et al., 1999).

McGinn et al. (2006) used a bLS dispersion technique where plume gas concentrations are measured several hundred meters downwind of a dairy farm. The bLS dispersion technique is a useful approach to measuring whole-farm emissions (e.g., dairies and feedlots) and emissions from well-defined point sources within the farm. In addition this technique is useful in evaluating mitigation strategies, is nonintrusive, less labor-intensive, and is much easier to implement than most techniques. Furthermore, using the bLS dispersion technique in conjunction with global positioning system (GPS) may have application for monitoring enteric CH_4 emissions from grazing cattle herds in large paddocks (McGinn et al., 2009).

Mitigation strategies to reduce enteric methane production

CH_4 mitigation strategies can be broadly divided into preventative and 'end of pipe' options. Preventative measures reduce carbon/nitrogen inputs into the system of animal husbandry, generally through dietary manipulation and, while a reduction in the volume of CH_4 emitted per animal may result, this is often secondary to the (primary) objective of improved productive efficiency. Alternatively, '*end of pipe*' options reduce or inhibit the production of CH_4 (methanogenesis) within the system of animal husbandry (Sejian et al., 2011a). Any reduction strategies must be confined to the following general framework viz., development priority, product demand, infrastructure, livestock resource and local resources. The most attractive emissions mitigation projects must balance the needs in all of these areas, so that no one factor creates a constraint on continued improvement in production efficiency, and the resulting CH_4 emissions reductions. Within this framework, CH_4 emissions mitigation options for enteric fermentation can encompass a wide range of activities across these areas. However, underlying these activities must be specific options for improving the production efficiency of the livestock. Without these options, CH_4 emissions cannot be reduced. The technologies that can reduce the amount of CH_4 production in rumen or total release of methane into atmosphere are useful for efficient use of feed and making the environment more favourable. Several options have been considered for mitigating CH_4 production and emitting in atmosphere by the livestock. All approaches point towards either reduction of methane production per animals or reduction per unit of animal product. There are several factors which need to be considered for selection of best options for CH_4 emission reduction: these include climate, economic, technical and material resources, existing manure management practices, regulatory requirements etc. Generally the CH_4 mitigation strategies can be grouped under three broader headings viz., managemental, nutritional and advanced biotechnological strategies (Sejian et al., 2011a). Figure 2 summarize the salient enteric methane mitigation strategies.

Managemental strategies

Animal manipulation by reducing livestock numbers: The countries which are committed to reduce the enteric methane emission from the livestock, reducing animal number is the best possible way but it is totally unacceptable for those countries which solely depend on livestock production for their national economic income (Sejian et al., 2011a). Shifting of old age cattle from heifers in the cattle herds can efficiently increase the productivity and decrease the

enteric methane production due to high intake and passage rate of ingested feed materials that can lead to lower enteric emission. Hegarty (2001) points out that if animal numbers do not decrease in response to the improved productivity, then emissions from the sector will increase rather than decrease. Sheep population has been reduced from 57.9 million in 1990 to 45.2 million in 2000, while dairy cattle and beef cattle population have increased slightly. The net outcome was a decline in ruminant CH_4 emission from 1.45 to 1.31 Tg/year from 1990 to 2000 (Sejian et al., 2011a). The application of biotechnological aids can meet out the loss of reduced animal number, for example, the use of recombinant bST (Bovine Somatotropin) leads to an increase in milk production upto 10-20% and therefore animal number can be reduced to lower the total enteric emission (Johnson et al., 1996; Clemens and Ahlgrimm, 2001). Kirchgessner et al. (1995) estimated that overall CH_4 emissions could be decreased by reducing animal numbers while maintaining milk production.

Animal breeding with low CH_4 emissions: Genetic selection of animals that consume less feed or produce less CH_4 per unit of feed is a management strategy that may be used to reduce enteric CH_4 emissions. Pinares-Patino et al. (2007) established that there are differences between individual animals in the quantity of CH_4 they emit per unit of dry matter intake. This finding has resulted in the establishment of research programmes aimed at exploiting these differences. Animal effects on fermentation could be via the saliva, feed processing (e.g., comminuition), or flow rate through the rumen. It is possible that the animal's impact on fermentation is genetically determined and if this is the case it may be possible to obtain markers that can be used to select low methane emitters.

Increasing the efficiency of livestock production: Improvement in the efficiency of ruminant animal performance will generally lead to a reduction of CH_4 emitted per unit of animal product. There are two aspects of this: genetic improvement of the animals themselves to achieve more product per unit of feed intake, as has been achieved with pigs and poultry and nutritional manipulation via increased feed intake and appropriate feed composition.

Increasing feed intake: Increasing feed intake decreases the methane emission per unit of feed intake. Kirchgessner et al. (1995) reported that as milk yield increases methane emitted per unit of milk yield decreases. By feeding animals *ad libitum* it is possible to both maximize efficiency and reduce CH_4 emission per unit of product. This is because as intake increases the methane emission associated with the essential, but non-productive, requirements for maintenance is diluted. By improving animal production efficiency, emissions per unit product can be reduced by 25 to 75% depending on animal management practices (Bowman et al., 1992). In addition, improved productivity can allow managers to reduce the size of the herd necessary to produce a certain quantity of product (O'Mara, 2004).

Nutritional strategies: As described above, decreasing dietary fibre and increasing starch and lipid will reduce methane emission. Generally, diets of higher digestibility have these characteristics. Generally dairy cows were given feeds of increasing digestibility to achieve the same level of milk production. The animals would have eaten less of the higher digestibility diets and thus produced less total methane and reduced methane emitted per unit of milk produced. Improving the nutritive value of the feed given to grazing animals by balancing the diet with concentrates, or by breeding improved pasture plants, should result in reduced methane emission.

Metabolic efficiency by production enhancing agents: Production enhancing agents are available for use to increase production efficiency in cattle. Bovine somatotropin (bST) for dairy cows is a naturally occurring growth hormone produced by the pituitary gland. Recombinant bST, an identical molecule, is produced biotechnologically and has been shown to increase milk production in US dairy cows. In general, the use of bST leads to an increase in milk production of 1020%, and therefore animal numbers can be reduced to lower total enteric emissions (Johnson et al., 1996; Clemens and Ahlgrimm, 2001). Johnson et al. (1996) estimated that the use of bST to improve US dairy cattle productivity could result in decreased CH_4 emissions (% of GEI) by about 9%.

Grazing management: Implementing proper grazing management practices to improve the quality of pastures will increase animal productivity and lower CH_4 per unit of product. McCaughey et al. (1997) observed that CH_4 production was greatest for steers continuously grazing at low stocking rates (1.1 steer ha^{-1}; 306.7 L d^{-1}) and least for steers grazing continuously at high stocking rates (2.2 steers ha^{-1}; 242.2 L d^{-1}). At higher stocking rates, forage availability and intake are low. When pastures were rotationally grazed, stocking rates had no effect on CH_4 production (McCaughey et al., 1997). At low stocking rates (1.1 steer ha^{-1}), CH_4 production (L ha^{-1} d^{-1}) was 9% lower on rotational grazing than continuous grazing (McCaughey et al., 1997). Measurements of CH_4 production from grazing beef cows indicated a 25% reduction in CH_4 losses with alfalfa-grass pastures (7.1% of GEI) compared to grass-only pastures (9.5% of GEI) (McCaughey et al., 1999). Early grazing of alfalfa-grass pastures, reduced CH_4 production (% GEI) by 29-45% in steers compared to grazing at mid and late seasons (Boadi et al., 2004).

Improved grassland and rangeland management: Increasing the digestibility of cell walls in forages has been suggested as a means to lower CH_4 losses, but in fresh grass and grass silage the scope of this approach appears to be rather limited. There is evidence that fresh grass results in lower CH_4 losses than grass silage, but no direct comparisons exist between fresh grass and grass silage. Mainly forage diets are often supplemented with sugar-based concentrates to provide a rapidly available source of energy for the rumen microbes or to increase the palatability of the diet DM and hence stimulate the digestibility (Mills et al., 2001). CH_4 production in ruminants tends to increase with maturity of forage fed, and CH_4 yield from the ruminal fermentation of legume forages is generally lower than the yield from grass forages (Moss et al., 2000). Shifting the animals from grass to legumes plant species tend to decrease the enteric emission due to lower proportion structural carbohydrates and faster rate of passage which shift the fermentation pattern towards higher propionate production (Johnson and Johnson, 1995). In New Zealand, Friesian and Jersey dairy cows grazing sulla (*Hedysarum coronarium*), a condensed tannin-containing legume, emitted less CH_4 per unit of DMI (19.5 g CH_4 kg DM^{-1}) compared to cows grazing perennial ryegrass pasture (24.6 CH_4 kg DM^{-1}) (Woodward et al., 2002). Johnson et al. (1996) concluded that altering the dietary cation anion balance of a roughage diet could decrease ruminal CH_4 production without altering other aspects of rumen fermentation. Continuous grazing of improved pasture managed with a high stocking rate (2.2 steers ha^{-1}) resulted in 21% lower daily CH_4 emissions as compared to the low stocking rate (1.1 steers ha^{-1}) when measured as emissions per animal per day, but these differences were not evident when measured as emissions per unit gain or as % GEI (McCaughey et al., 1997).

Longevity / extended lactation: It can reduce the energy demand of cows and methane by approximately 10%. Extended lactation has other benefits, such as reducing peak workload; cow health problems (due to less calving) and less heifer replacements are required. Milk in the extended lactation phase is higher in milk solids, making the milk more valuable per litre and there may be price incentives for milk produced outside peak supply months. Extended lactation is always considered as an option for herd, and selected breeds suited for extended lactation. The longer the cows stay in a herd, the lower the number of replacements required, and thus the lower the total farm methane emissions. An example of a 100 cow farm is where the average number of lactations varies from 2.5 to 5. It is assumed that dairy cow emissions are 118 kg/yr while the rearing of a replacement heifer to calve at 2 years old results in methane emissions of 100 kg. This shows that total farm emissions of CH_4 from enteric fermentation decline from 15,800 kg/yr to 13,800 kg/yr (0.127 less) as the average number of lactations increases from 2.5 to 5. This does not factor in the higher yield of the older cows which would further reduce emissions per kg of milk. Thus any measures which reduce involuntary culling should be encouraged.

Nutritional strategies

Dietary manipulation: The chemical composition of diet is an important factor which affects rumen fermentation and methane emission by the animals. Methane production was significantly lower in the sheep fed on green sorghum and wheat straw in the ratio of 90:10 as compared to where the ratio was 60:40 (31.5 vs 46.91/kg). Improvement in the digestibility of lignocellulose feeds with different treatments also resulted in lower methanogenesis by the animals (Agrawal and Kamra, 2010). Wheat straw treated with urea (4kg urea /100kg DM) or urea plus calcium hydroxide (3kg urea+3 kg calcium hydroxide /100kg DM) and stored for 21 days before feeding, reduced methane emission from sheep. The treatment of straw with urea and urea molasses mineral block lick caused a reduction of 12-15% methane production and the molar proportion of acetate decreased accompanied with an increase in propionate production (Agrawal and Kamra, 2010). On inclusion of green maize and berseem in the ration, methanogenesis decreased significantly. By increasing the concentrate level in the paddy straw based diet there was a depression in methane production accompanied with an increase in propionate concentration in the rumen liquor. Castor bean cake and karanj cake inhibited methanogenesis significantly, but these two oil cakes also affected in *vitro* dry matter degradability of feed adversely, which might be due to the presence of anti-nutritional factors (Kumar at al., 2007). Fumaric acid is a precursor of propionic acid in the fermentation of feed in the rumen and can act as an alternate sink for consumption of hydrogen generated in the rumen. The levels of fumaric acid required to inhibit methanogenesis to a significant extent may cause a drop in pH which might affect feed fermentation adversely. Free fumaric acid (10% in the ration) and an equivalent amount of encapsulated fumaric acid decreased methane emission to the extent of 49% and 75% compared to control sheep without supplementation of fumaric acid (Agrawal and Kamra, 2010).

Increased proportion of concentrates in the diet: A higher proportion of concentrate in the diet leads to a reduction in CH_4 emissions as a proportion of energy intake (Yan et al., 2000). The relationship between concentrate proportion in the diet and methane production is curvilinear (Sauvant and Giger-Reverdin, 2007) with a marked decrease in methane observed when dietary starch is higher than 40%. Replacing plant fibre in the diet with starch induces a shift of VFA production from acetate towards propionate occurs, which results in less hydrogen production (Singh, 2010). A positive response to high levels of grain based concentrate on methane reduction has also been reported by others (Beauchemin and McGinn, 2005; McAllister and Newbold, 2008). The metabolic pathways involved in hydrogen production and utilization and the activity of methanogens are two important factors that should be considered when developing strategies to control methane emissions by ruminants (Sejian and Naqvi, 2011b). Reduction of hydrogen production should be achieved without impairing feed fermentation. Reducing methanogens activity and/or numbers should ideally be done with a concomitant stimulation of pathways that consume hydrogen to avoid the negative effect of the partial pressure increase of this gas. Many mitigating strategies proposed have indeed multiple modes of action (Martin et al., 2008). Hydrogen gas produced during microbial fermentation of feed is used as an energy source by methanogens, which produce methane. Efficient H_2 removal is postulated to increase the rate of fermentation eliminating the inhibitory effect of H_2 on the microbial degradation of plant material (McAllister and Newbold, 2008). The rate of CH_4 formation is determined by the rate at which H_2 passes through the dissolved pool, and the amount of CH_4 formed is determined by the amount of H_2 that passes thought the pool. The absolute amount of CH_4 formed per animal on different diets is related to characteristics of the feed in complex ways including the nature and amount of feed, the extent of its degradation and the amount of H_2 formed from it (Singh, 2010).

Adding lipid to the diet: Dietary fat seems a promising nutritional alternative to depress ruminal methanogenesis without decreasing ruminal pH as opposed to concentrates (Sejian et al., 2011b). Addition of oils to ruminant diets may decrease CH_4 emission by up to 80% *in vitro* and about 25% *in vivo* (Singh, 2010). Lipids cause depressive effect on CH_4 emission by toxicity to methanogens, reduction of protozoa numbers and therefore protozoa associated methanogens and a reduction in fibre digestion. Oils containing lauric acid and myrstic acid are particularly toxic to

methanogens. Beauchemin et al. (2008) reviewed the effect of level of dietary lipid on CH_4 emissions over 17 studies and reported that with beef cattle, dairy cows and lambs, for every 1% (DMI basis) increase in fat in the diet, CH4 (g/kg DMI) was reduced by 5.6%. In another review of fat effects on enteric CH_4 compared a total of 67 *in vivo* diets with beef, sheep and dairy cattle, reporting an average of 3.8% (g/kg DMI) less enteric CH_4 with each 1% addition of fat (Singh, 2010).

Ionophores: Ionophores (e.g. monensin) are antimicrobials which are widely used in animal production to improve performance. Tadeschi et al. (2003) reported in a recent review that on feedlot and low forage diets, tend to marginally increase average daily gain whilst at the same time reducing DMI, thus increasing feed efficiency by about 6%. Monensin should reduce CH_4 emissions because it reduces DMI, and because of a shift in rumen VFA proportions towards propionate and a reduction in ruminal protozoa numbers (Singh, 2010). *In vivo* studies have shown that animals treated with monensin emit reduced levels of CH_4 (McGinn et al., 2004; van Vugt et al., 2005) but others have reported no significant effect (Waghorn et al., 2002). Monensin causes a direct inhibition on H-producing bacteria that result in a decrease in methane production due to shortage of molecular hydrogen. Monensin also favours propionate producing bacteria.

Plant secondary metabolites: The term plant secondary metabolite is used to describe a group of chemical compounds found in plants that are not involved in the primary biochemical processes of plant growth and reproduction (Agrawal and Kamra, 2010). These compounds might function as a nutrient store and defense mechanisms which ensures survival of their structure and reproductive elements protecting against insect or pathogen predation or by restricting grazing herbivores. Several thousand of plant secondary metabolites have been reported in various plants and many of them have found their use in traditional Indian and Chinese system of medicine (Kumar et al., 2007).

Saponins: Numerous studies have demonstrated that saponins and saponin-containing plants have toxic effects on protozoa. Forages containing condensed tannins have been shown to decrease methane production by the ruminants. Tannins present in C*alliandra calothyrus* reduced nutrient degradation and methane release per gram of organic matter degraded in *in vitro* experiments with rumen simulation technique (RUSITEC). Woodward et al. (2002) investigated the effect of feeding of sulla on methane emission and milk yield in Friesian and Jersey dairy cows. Cows feed sulla produced less methane per kg DM intake (19.5 vs. 24.6 g) and per kg milk solid yield (243.3 vs. 327.8 g). Similar trends in methane emission and milk production have been observed in sheep fed on lotus silage (Woodward et al., 2001) there was also 16% reduction in methane production in lambs fed on *Lotus pedunculatus* (lotus), which might be due to the presence of condensed tannins (Waghorn et al., 2002). Another condensed tannins containing forage *Sericea lespedeza* (17.7% CT) decreased methane emission (7.4 vs. 10.6 g/d and 6.9 vs. 16.2 g/kg DMI for *Sericea lespedeza* and crabgrass /tall fescue, respectively) in angora goats (Puchala et al., 2005; Agrawal and Kamra, 2010). *Bergenia crassifolia, Emblica officinalis, Peltiphyllum peltatum, Populus deltoids, Quercus Incana, Rheum undulatum, Terminalia belerica, Terminalia chebula* and *Vaccinium vitis-idaea* are some other plants containing high tannin contents and have a potential to inhibit *in vitro* as well as *in vivo* methane emission by the rumen microbes (Patra et al., 2006; kumar et al., 2009).

Essential oils: *Allium sativum, Coriandrum sativum, Eucalyptus globules, Foeniculum vulgare, Mentha piperita, Ocimum sanctum, Populus deltoids* and *Syzygium aromaticum* are some of the plants which contain high concentration of essential oils and are effective against methane emission and protozoa growth in the rumen, but some of them also have adverse effects on degradability of feed and nutrient utilization by the animals. The results of in vivo experiments with these plants are also variable and need further experimentation before their practical application in the livestock production (Agrawal and Kamra, 2010).

Bacteriocins: Some bacteriocins are known to reduce methane production *in vitro* (Callaway et al., 1997; Lee et al., 2002). Nisin is thought to act indirectly, affecting hydrogen producing microbes in a similar way to that of the ionophore antibiotic monensin (Callaway et al., 1997). A bacteriocin obtained from a rumen bacterium, bovicin HC5, decreased methane production *in vitro* up to 50% without inducing methanogens adaptation (Lee et al., 2002).

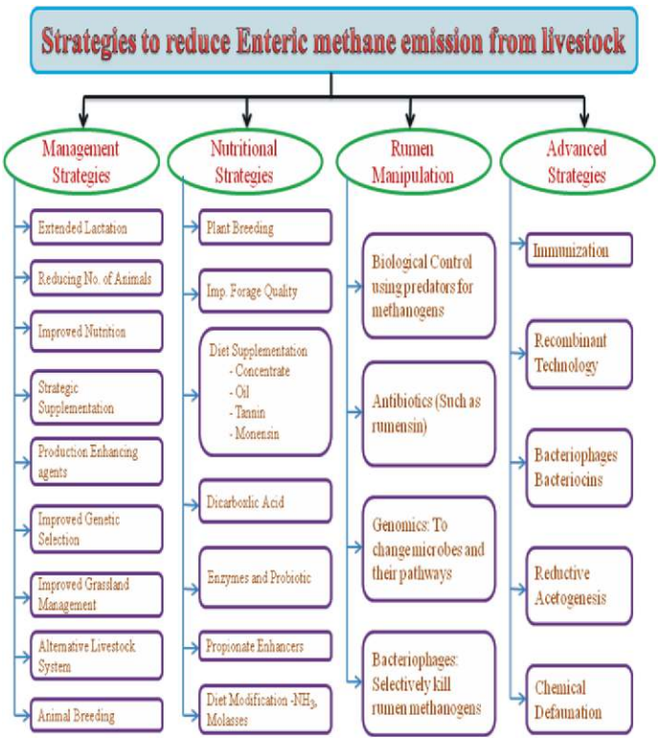


Fig. 2 Salient mitigation strategies to reduce enteric methane emission

Organic acids: Organic acids are generally fermented to propionate in the rumen, and in the process reducing equivalents are consumed. Thus they can be an alternative sink for hydrogen and reduce the amount of hydrogen used in CH_4 formation. Newbold et al. (2005) reported fumarate and acrylate to be the most effective in batch culture and artificial rumen. There have been some recent *in vivo* studies. Newbold et al. (2002) reported a dose-dependent response to fumarate in sheep. Wallace et al. (2006) described a proportional reduction of 0.40-0.75 when encapsulated fumaric acid (0.1% of diet) was fed to sheep. While the level of reduction in CH_4 emissions that could be achieved is somewhat uncertain, the main impediment to this strategy is the current cost of organic acids which makes their use uneconomical. Integrated research investigating animal, plant, microbes and nutrient level strategies might offer a long term solution of methane production. At the animal level, genetic selection is the area of research with the best chance of finding a solution. At the microbe level, vaccination and probiotics are the promising approaches for future research. Any mitigation strategy that reduces methanogen populations must also include an alternative pathway for H_2 removal from the rumen. Improvement and breeding of plants is another helpful way to control of methanogenesis, but the estimation of time required must be realistic. Strategies must however suit particular classes of livestock. Advances brought about through rumen metagenomic projects and the utilization of new technologies will broaden our understanding of the mechanisms involved in methanogenesis and other metabolic H_2 consuming and releasing processes, and will help find new tools for mitigation (Morgavi et al., 2010). The sustainability of methane

suppressing strategies is an important issue. There is an urgent need for support model that is capable of evaluating the effectiveness of both existing and new technologies for reducing methane emission.

Advanced biotechnological strategies

The most noteworthy achievement to reduce CH_4 production in livestock farms is the development of a vaccine containing an antigen derived from methanogenic bacteria (Gworgwor et al., 2006) and an immunogenic preparation which reduces the activity of rumen protozoa (Baker et al., 1997). Such vaccines have the potential to provide a cost-effective treatment to reduce CH_4 emission and enhance animal production. Baker (1995) and Shu et al. (1999) proposed that it may be possible to immunize ruminants against their own methanogens with associated decrease in CH_4 output and that such an approach can successfully reduce the members of streptococci and lactobacilli in the rumen..

Sheep production adapting to climate change

The sheep sector is relatively small, even when looked at as a global industry, but social contribution is huge, both in terms of the production of a quality, healthy red meat and through the use of sheep as a positive environmental management tool. Promotion of sustainable sheep production will be vital to ensure that the impact of climate change is minimized on the sheep farmers. This will involve rearing of animals which are more sturdy, heat tolerant, disease resistant and relatively adaptable to the adverse conditions. In such a situation some of the indigenous breeds will be able to cope much better than the crossbred as crosses containing higher exotic inheritance exhibiting problems of survival when compared to indigenous breeds.

Relatively, there is a lot of basic knowledge on the interaction between heat stress and sheep production, reproduction and health traits. The implementation of the knowledge to maintain the welfare of animals maintained under extensive management systems is difficult because of objective limitations to monitor heat stress and economic compulsions in applying measures to ameliorate heat stress. From welfare point of view, ideally the animals should be raised in the zone of optimal thermal well being. However, these would be almost impracticable goal to attain in dominant grazing system of the world. The following recommendations are general rules that can be applied under extensive conditions:

- Provision of shade shelter in areas where typical ambient temperature during summer exceeds above than normal
- Provision of water: it is recommended that the distance between watering spot and grazing area be such that grazing sheep are able to visit the water spot at least once a day. In order to avoid negative interactions with other stressful factors, particular emphasize should be given for adequate supplementation and provision of clean water.

In extensive system of rearing generally, sustainable sheep production is pasture-based and requires little or no supplemental feed. The ways and means by which sustainable sheep production can minimize climate change are:

- Producing forage on-site and without the use of energy-intensive inputs including fertilizers, herbicides, and fuels to dry and store feed, generally lowers the embodied energy in sheep feed
- When feeding native hay and grains that are produced locally, the energy required for transportation is reduced further due to shorter distances between the feed source and the sheep
- Since fossil fuels are primary sources of GHG emissions such as CO_2, using fewer energy inputs usually reduces emissions as well

- Providing sheep with access to pasture forage improves the ecological balance between forage and livestock
- Pastured sheep efficiently close the loop between harvesting forage and returning nutrients to the soil, and with less energy than if forage were harvested and hauled from the pasture and manure was then hauled back out onto the pasture
- Distributing manure and urine on the pasture also reduces methane emissions from manure slurry
- Proper soil and pasture management can also mitigate the release of emissions. Under certain soil conditions, N_2O emissions are released from the soil through a process called de nitrification. An excessive buildup of manure and urine (nitrogen, ammonium) in water-saturated soils can lead to de nitrification and the release of N_2O, a greenhouse gas 310 times more powerful than CO_2. Rotating animals through pastures and moving feeding, watering, and shade areas will help spread the manure and urine out more uniformly and may help decrease N_2O emissions from pasture soils.

As sheep is the ruminant animal, its contribution to global warming is by far through CH_4 production. Hence while aiming at sustainable sheep production; it is imperative to concentrate on reduction strategies for enteric methane production. The enteric methane emission reduction strategies can be grouped under three broader headings including managemental, nutritional and other molecular strategies. Any reduction strategies must be confined to the following general framework such as development priority, product demand, infrastructure, livestock resource and local resources. The most attractive emissions mitigation projects must balance the needs in all of these areas, so that no one factor creates a constraint on continued improvement in production efficiency, and the resulting CH_4 emission reductions. Within this framework, CH_4 emissions mitigation options for enteric fermentation can encompass a wide range of activities across these areas. However, underlying these activities must be specific options for improving the production efficiency of sheep. Without these options, CH_4 emissions cannot be reduced.

Future strategies to sustain sheep production under changing climate scenario

- Conduct a thorough review of the impact of sheep farm production on climate change and their wider societal impacts, including the impacts of the various types of production systems (e.g., extensive, industrial, indigenous, intensive).
- Acquire consultation and guidance from welfare scientists and experts when drafting climate change policy, such as how to reduce green house gases emissions, agriculture management, mitigation strategies and disaster response to different climate change scenarios and include strategies for minimizing the risk to animals from climate change.
- Investigate the outcomes of shifting to more sustainable sheep production systems, such as organic or semi-intensive and the related effects on GHG emissions.
- Include strategies on husbandry (feed, genetic makeup, lifespan), management system (organic, water-intensive extensive, housed) and outputs (manure) for cutting emissions on global, national, and regional scales.
- Encourage low-intensity/density farming system policies and strategies.
- Sheep sector GHG emission can be considerably reduced by increasing the plane of nutrition.
- Shifting rearing system from extensive to semi-intensive/intensive system is a positive approach to reduce GHG emission. This can be achieved by providing high plane of nutrition and supplementation of high quality forage.

- Identification and characterization for genes to adapt to drought and heat stress in sheep.
- Developing resilient crop-livestock production systems through better soil and water management and higher water-use efficiency.
- Improved livestock capacity to cope with climate change through the identification and improvement of local breeds adapted to the local feed resources and tolerant to heat / cold stress.
- Develop sustainable adaptation techniques and farming strategies in collaboration with farmers, agriculture extension agents, women's groups, farm animal welfare experts and advocates, and political bodies.

Conclusions

Scientific research can help the livestock sector in the battle against climate change. All animal scientists must collaborate closely with colleagues of other disciplines, first with agronomists, then physicists, meteorologists, engineers, economists, etc. The effort in selecting animals that up to now has been primarily oriented toward productive traits, from now on, must be oriented toward robustness, and above all, adaptability to heat stress. In this way, molecular biology could allow to directly achieve genotypes with the necessary phenotypic characteristics. Research must continue developing new techniques of cooling systems such as thermo-isolation, concentrating more than in the past on techniques requiring low energy expenditure. New indices that are more complete than THI (Thermal-Humidity Index) to evaluate the climatic effects on each animal species must be developed and weather forecast reports must also be developed with these indices, to inform the farmers in advance. Above all to beat the climate change or in any case not to let the climate beat livestock systems, researchers must be very aware of technologies of water conservation.

Adaptation to climate change is an integral part of agricultural production now and will become more important in the future as the impacts of climate change become more evident. In developing a strategy for adapting to climate change, one key challenge is dealing with uncertainty. Significant uncertainty relates to the nature and extent of regional climate change impacts, impacts across agricultural industries, and impacts over time. The challenge for governments and agricultural industry stakeholders is to deal with these uncertainties through further research and the development of policies and farm management approaches that are flexible enough to deal effectively with a range of potential climate change outcomes.

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Resistance to Gastrointestinal Nematodes in Sheep

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Sheep is popularly known as 'Museum of Parasite' due to their grazing habit. Gastrointestinal nematodes (GIN) are among the most important infections faced by livestock (particularly small ruminants) and have impact on their production, as well as on livestock welfare and robustness. Across host species, helminthosis was the top ranking disease followed by neonatal mortality, FMD and ectoparasitism (Davis et al., 2009). Animal well being has become a significant concern among consumers who expect food animals to be well treated and free of diseases. Consumers also expect their meat products to be free of residual drugs. Disease costs are estimated to account for 10-20% of turnover in developed countries. In the US, economic losses in ruminants due to parasites are estimated at more than $ 3 billion per year. In sheep they are estimated up to 60% of all economic losses occurring in this species (Kloosterman et al., 1992). The total annual cost of parasitism to be estimated as $ 222.0 million to the Australian sheep industry (McLeod, 1995), while in USA, the economic losses in ruminants due to parasites are estimated at more than $ 3.0 billion per year (Smith, 2002). It is considered that the cost of GI parasites will be $700 million by 2010 as sheep numbers are reduced in response to increased worm resistance to drenches (Welsman, 2001). Within sub-Saharan Africa, de Haan and Bekure (1991) estimated that endoparasites cause mortality and production losses to the order of $ 2.0 billion per annum. McLeod (2004) estimated annual costs of roundworm parasites in selected Asian countries and found that roundworm-inflicted production losses to sheep production were at the maximum of $ 5.6 million / year in Indonesia followed by Nepal and Philippines. Substantial annual economic losses to the tune of $ 103.0 million and $ 111.0 million were estimated to be inflicted by roundworm infection in small ruminants of India and Australia, respectively (McLeod, 2004). Gazehegan (1992) estimated a loss of US$ 400 million due to helminthosis in Ethiopian meat industry. With the approach of partial farm budgeting losses caused by GIN in sheep flocks in Rajasthan, Singh et al. (2011) estimated that under natural challenge of infection, the overall annual loss per adult and yearling sheep was Rs. 125.78 and Rs. 94.29 per head, respectively. Based on sheep population (Census, 2003) the total annual loss in Rajasthan was estimated to be Rs. 1191.71 million. The contribution to loss was comprised of losses in mutton production (59.56%), increased susceptibility for mortality (16.57%), premature culling (11.25%), reduced fertility (7.97%) and decreased wool yield (4.65%). Cost-benefit analysis exhibited that single strategic anthelmintic intervention during mid to late monsoon avoids losses by 45.53 to 59.00% (Singh et al., 2011).

For parasitic gastroenteritis, the problem is serious, as chemotherapy is becoming less effective due to rampant rise in emergence of anthelmintic resistance in parasites to chemicals (Singh et al., 2002; Singh and Swarnkar, 2008; Swarnkar and Singh, 2010). Furthermore, prospects for vaccination are not encouraging. This situation largely reflects absence or un-sustainability of worm control measures. For these reasons, new alternatives to addressing problem of GIN are needed. Recent advances in animal genomics and immunology and in the understanding of host-pathogen interactions provide new opportunities to develop more effective control strategies. Where conventional control measures, such as vaccination and chemotherapy, have been either ineffective, unsustainable or uneconomic, generic approaches to disease control have been considered. It has been well established that rarely will all animals in a population, when exposed to an infectious disease, exhibit clinical symptoms. However, it is difficult to determine why some animals become sick while others remain healthy. Animal health is influenced by several factors like genetics, nutrition, age, stress, management system, season, pathogen dosage, immunological status, epidemiology and many other variables. These factors interact, thus confounding our ability to understand the mechanisms of disease resistance.

Evolutionary theory suggests that genetic diversity and resistance to parasites are linked (Howard and Lively 1998). Individuals with low genetic diversity may be less able to cope with parasite infection or lack the same range of parasite resistance mechanisms carried by more heterozygous species (Coltman et al., 1999). Loss of genetic diversity coupled with increased disease may be a crucial mechanism driving population extinction risk (Luikart et al., 2008) . Disease resistance is a particularly important attribute of livestock in low-input livestock production systems in the developing world (Bishop et al., 2002). Resistance to infectious diseases is often the critical determinant of the sustainability of such systems, and improving resistance is perceived as a primary target for genetic improvement programmes. Evidence that domestic animals show variation in the ability to resist infection with parasites has been documented since the beginning of the 20th century (Stear and Wakelin, 1998). Such variation in response is now known to occur both within and between breeds of livestock (Owen and Axford, 1991). Systematic analysis of variation in resistance to parasites began in the 1950s and has now become a major area of research (Wakelin and Blackwell, 1993). The work has illuminated many aspects of the innate and acquired immune response to parasites, has contributed to fundamental insights into immunological mechanisms and has provided the basis for alternative approaches to the control of parasitic infections in domestic animals. An enhanced capacity to resist infection is clearly inherited and can be passed from parent to offspring, usually as a dominant trait (Wakelin and Blackwell, 1988).

Evidence for genetic variation amongst sheep in their resistance to GINs is well documented in many breeds (Gasbarre and Miller, 2000; Bishop and Morris, 2007). Breeding programs for selecting commercial animals for enhanced resistance against nematode parasites are used for meat and wool breeds (Nieuwhof and Evans, 2003). Nematode resistance has moderate heritability and selection lines of sheep with large differences in faecal egg count (FEC) between resistant and susceptible lines have been generated. This resistance pertains to a variety of nematode species such as *Haemonchus contortus* (Woolaston and Piper, 1996), *Trichostrongylus colubriformis* and *Ostertagia* species (Morris et al., 2000). Attempts have been made to identify susceptible and resistant animals based on indicator traits like fecal egg counts (FEC), packed cell volume (PCV), circulating eosinophils, lymphocytes responsiveness, ovine lymphocyte antigens types, immunologenic response to larval antigen and circulating antibodies in several laboratories. Although, these indicator traits have certain limitations to accurately select the animal for resistance to internal parasites. Selecting for resistance would be substantially simplified if the differences between resistance and susceptibility were known at the gene variant level and animals could be selected based on genotype. In recent years, research on the MHC as candidate genes of disease resistance and susceptibility has become a major focus in animal breeding. There are attempts at identifying QTL(s) /gene(s) of resistance to GINs allowing selection for resistance without expensive and wasteful of animals testing for nematode infestation. The outcome of research on disease resistance has brought technological advances which will allow farmers to breed flocks with increased disease resistance and to integrate such programmes in an integrated flock-health control strategy (Raadsma et al. 1998). India with its huge genetic diversity in sheep has potential to manipulate the gene pool for control of parasites, although it has not been exploited so far. This review will focus primarily on variation in responses to GI nematode parasites and will deal first with some general principles of resistance and on the selection of animals for increased genetic resistance to disease, leading to better healthy, productive and robust animals.

Definitions: When considering disease resistance, defining the most appropriate traits to allow description of resistance in a breeding programme and to allow selection of the desired individuals is critical. Disease resistance itself takes many forms like resistance to infection *per se*, ability to limit proliferation and transmission of parasites / pathogens and minimal occurrence of disease (tolerance).

Resistance: The fundamental definition of 'disease resistance' includes freedom from clinical signs of disease after challenge. Resistant animals remain free from clinical signs of disease, whereas susceptible animals become

affected after being subjected to the same disease challenge. A notable departure from the above definition applies to internal parasites. Animals may be termed 'resistant' to a given infection if, after entry into the body, the parasite:

- Fails to establish an infection
- Establishes an infection but then fails to complete development
- Establishes and develops infection but is then controlled or eliminated by the host

Resilience: Hosts suffering little or no adverse effects on productivity during infection are defined as 'resilient'. As a breeding goal, resilience has the advantage that no direct measures of the relative pathogen loads of animals are necessary. A significant disadvantage, however, is that true resilience is impractical to measure, because costly and time-consuming measurements of production are necessary. Resilient hosts, unlike their resistant counterparts, may have no favourable effect on the concentration of the pathogen, such as at the level of pasture contamination. Traits defined specifically to describe resilience to internal parasites have been found to have low heritability (Albers et al., 1987) and usually require a prolonged period of infection, which is generally unacceptable to breeders of elite animals.

Risk of parasite evolution

In host-parasite relationship, long evolutionary has produced a rich and complex series of co-adoptions by host and parasites (Stear et al., 2009). The fundamental theorem of natural selection suggests that evolution will fix genes / alleles that improve fitness (Fisher, 1930). For example, gene variants or alleles which are related to parasite resistance should mainly be found, or at least be at a higher frequency, in breeds which originate from regions with high parasite burden because of high selection pressure. In majority of developed countries, this natural pressure was reduced drastically with the advent of anthelmintics about seven decades ago. However, in large part of the tropical developing world, where little or no anthelmintics are used, the natural selection pressure still exists today. A common question is whether or not the parasite will evolve to overcome the genetic changes in the host. Absolute risks of parasite evolution are not easily estimated for any disease control intervention; the most important question is whether parasite evolution is more or less likely when genetic control strategies are used rather than other strategies? To answer this question, two types of genetic improvement can be identified:

- Utilization of resistance mechanisms that have evolved in indigenous breeds of livestock subject to endemic disease challenge for hundreds or thousands of years. It has a high likelihood of having long-term sustainability and will be the application of choice where feasible.
- Selection of disease resistance genes of unknown origin. These are more likely to represent relatively new mutations that have not been tested by natural selection for their effect on the evolution of the pathogen, the outcome of the genetic improvement will be less certain. Aspects of these risks have previously been considered by Bishop and MacKenzie (2003).

Sustainability of genetic resistance: Some key factors pertaining to the sustainability of genetic resistance are as follows:

- Pathogens with large population sizes and short generation intervals have the greatest potential to evolve in ways that defeat host disease resistance. Thus, host genetic resistance could be more sustainable for macroparasites such as nematodes than for viruses and bacteria.
- Disease control strategies that combine different approaches will generally be more sustainable, as parasites with a mutation allowing them to escape one strategy will still be susceptible to other forms of control. Thus, the combined use of host genetic resistance with other control strategies will often be more

sustainable than use of any one control strategy alone. Also, host genetic resistance based on several genes will often be more sustainable than resistance based on a single gene.

- Genes that cause host resistance will place a greater selection pressure on the pathogen to evolve than those for host tolerance. Risks are less if the resistance mechanism is reduced susceptibility to infection than if the mechanism is control of pathogen population growth or transmission.
- Selection pressures on the pathogen caused by host genetic resistance will usually be lower than with therapeutic or vaccine interventions. Therefore, host genetic resistance should be more sustainable than disease control interventions that place a strong selection pressure on successful parasite mutants.
- Genetic selection for improved disease resistance can be based directly on disease phenotype, on indicators of the state of the disease or on genetic markers for genes that cause disease resistance. Arguably, with genetic markers there is a danger that parasite evolution may go unnoticed and marker-based selection may be more risky. In practice, the greatest pressure on the pathogen to evolve will only occur after genetic improvement is widely disseminated in the livestock production system, so that use of molecular markers probably does not create a significantly greater risk of pathogen evolution than other methods of selection.

Immunological expression of parasitic resistance

Knowledge of the mode of disease infection and host response is essential to comprehend the complexity of selecting for disease resistance. In general, the pathogen must be present in the host's environment and it must penetrate host cell barriers in sufficient numbers, attack target cells and replicate. The host has three immune defences against infection: natural, innate, and acquired immunity. To maintain health all three must be present and functioning.

Natural immunity is the first barrier and is comprised of skin, hair, mucous membranes, secretions (tears, urine, stomach, saliva, mucous, skin secretions, etc.), grooming behavior (licking, dust rolling, tail swishing, etc.) and favorable microorganisms that compete directly or indirectly against pathogens. There are also nutritional components to natural immunity. Dehydration and malnutrition can decrease natural secretions making some tissue more susceptible to infection. Vitamin and mineral deficiencies result in suppressed immune systems. Innate and acquired immunity are co-dependent and form a complex network of cells and tissues that interact to detect and attack pathogens or associated antigens. The innate immunity refers to the immune system one is born with and is the initial response by the body to eliminate microbes and prevent infection. It commonly involves white blood cells (natural killer cells, neutrophils, eosinophils, monocytes, and macrophages), complement proteins (C1 - C4) that adhere to pathogens, and cytokines (interferons and chemokines) that attract immune cells to the site of infection. The innate immune system constantly searches for antigens (bacteria, fungi, and viruses). When an antigen is discovered, the innate system can attack it or illicit inflammation to attract immune cells. The innate system is not specific to any one type of pathogen and has no memory of previous exposure to a pathogen or antigen.

The acquired immune system is developed from previous exposure to pathogens or vaccines and can recognize pathogens previously exposed to. Acquired immunity is antigen specific. There are two types of acquired immunity: the cell-mediated immunity is comprised of immune cells that directly attack pathogen infected cells, and the humoral immunity which is made up of antibodies (specific immune proteins) that are directed at the pathogens themselves. The acquired immune system is comprised of T and B cells, which are specialized white blood cells. The T cells destroy pathogen-infected cells. The B cells develop into specific antibody producing cells. Acquired immunity occurs in two forms: passive and active. Passive or maternal immunity is passed from the dam to the offspring via colostrum containing high levels of antibodies. Passive immunity is temporary. Disease resistance of very young lambs is highly dependent on passive immunity. This type of protection is short lived.

Numerous studies have shown that a proportion of genetic resistance to parasites in sheep is immunologically mediated. The cascade of immune phenomena developed against nematodes is complex and involves a large number of genes in the model systems where it has been studied (Garside et al., 2000). In ruminants, resistance to both infective larvae and adult nematodes has been associated with the development of acquired immunity (Balic et al., 2000). Thus, while spontaneous expulsion of a primary infection rarely occurs in sheep, it routinely occurs in previously primed animals although it varies considerably between individuals. This variation might be caused by different magnitudes of the same response mechanism, by the expression of different immune mechanisms or by a combination of both. Parasite rejection can be antigenic in nature but may also involve processes such as mechanical or enzymatic damage inflicted by the host on larval or adult parasites. Miller (1984) studied the traits like intestinal mucous antibody, serum and mucus mast cell protease and histamine, tissue eosinophilia, cytokine expression and lymphocytes surface phenotype with response to GINs. Accumulation of immune-mediated cell types (mucosal mast cells (MMC), globule leucocytes and eosinophils) in GI mucosa found to increase in resistant lines (Presson et al*.,* 1988; Gamble and Zajac, 1992; Gill et al., 1993).

Earlier studies have shown that genetic resistance of sheep to GIN is immunologically mediated (Gill, 1991). A variety of host responses including mucosal mast cell, globule leucocyte and eosinophil hyperplasia, and specific antibodies correlate with resistance (Gill, 1991). Studies in laboratory rodents have shown that protective immune responses induced by helminths are T-cell dependent (Mitchell, 1980) and that the CD4+ subset of T cells plays a major role in mediating protection against challenge infections with *Trichinella spiralis* and *Heligmosomoides polygyrus*,(Grencis et al. 1985; Urban et al. 1991) and spontaneous expulsion of *Nippostrongylus brasiliensis* during a primary infection (Katona et al. 1988). Gill et al. (1993) investigated the role of $CD4^+$ and $CD8^+$ T-cells in mediating resistance to *H. contortus.* It was observed that CD4+ T cells play a pivotal role in mediating genetic resistance to *H. contortus*, and in the generation of mucosal mast cell hyperplasia, tissue eosinophilia and anti-*Haemonchus* antibody. In contrast, depletion of CD8 + T cells had no effect on genetic resistance as faecal egg output, worm counts, mast cells and eosinophil responses in CD8-depleted lambs were not significantly different from those in controls. Thus, CD8+ T cells appear to play no protective role. However, in other study of Gill (1994) found no difference in worm burden, anti-larval antibodies, eosinophils and MMC in genetically resistant as well as in random bred following primary challenge with *H. contortus* infection.

Greater resistance to GIN infection is often associated with greater antibody responses, higher levels of T-cell proliferation and increased inflammatory responses, particularly those involving eosinophils and mast cells (Douch et al. 1996). Concerning the transcriptome studies, it seem that expression of TLR geners conducting O_2 and NO production, MHC, IgA and IgE production are important for resistance. But there are some discrepancies concerning a possible polarization of the TH1 / TH2 immune response that remains to be elucidated. Some studies show a resistance associated with a TH2 immune response, while other show association with a more balance response between TH1 and TH2. Bisset et al. (1996a) reported that Romney lambs selectively bred for resistance having significantly higher serum *T. colubriformis* specific antibodies, IgM and IgG1 than the susceptible lambs. Wakelin (1996) demonstrated role of interferon-gamma (IFN-) a cytokine, in the regulation of the immune response to parasitic infection. It is secreted by the T and NK cells, resulting in the activation of macrophages and general up-regulation of the cell mediated response via potentiating the Th1 cell response, while it down regulates the production of the Th2 cell subsets. The Th2 cells are associated with resistance to infection from intracellular gastrointestinal nematodes, while the Th1 response is relatively beneficial to the survival of the nematode parasite and detrimental to the host resistance (Coltman, et al., 2001). These studies show that parasite resistance has an immunological basis, the precise mechanism involved, which enables sheep to expel or control their worm burden, remain elusive.

Genetic variation in host resistance to parasite

It has been well established that the ability of animals to acquire immunity and express resistance against diseases varies substantially among and within breeds and is at least partly under genetic control (Bishop and Morris, 2007). Resistance to internal parasites such as *H. contortus*, *O. circumcincta and T. colubriformis* is under genetic control and subject is vividly reviewed by Wakelin, (1978), Barger, (1989), Stear et al., (1990), Baker et al., (1990, 1991), Gray and Gill, (1993), Raadsma et al., (1997) and Stear and Wakelin, (1998). Traditional breeding programmes have been used to establish the flock of sheep with high levels of resistance (Windon and Dineen, 1984; Albers et al., 1987; Windon 1990; Woolaston et al., 1991, Morris, 1998). Selection for disease resistance is costly. Potential costs associated with measuring disease resistance include reduced production, mortality, decreased longevity, diagnostic costs, and therapeutic expenses. Host differences in susceptibility to endoparasites have been the subject of genetic studies in sheep for over 30 years. Following examples from early cattle studies (Frisch 1981), research groups attempted to monitor host-genetic variation in nematode parasite burdens via breed differences, then via genetic variation among sire groups, and then exploiting it via experimental selection lines.

Several genes are involved in the control of resistance and variation between individuals is quantitative in nature, following a continuous distribution curve. Some individual sheep may carry genes which confer a qualitatively greater resistance to *H. contortus* than is seen in the rest of the population (Gray, 1987). There are many examples in which background genes are known to control quantitative variation in resistance to GI nematodes, and biologically these may well be the most important determinants of responses to infection. Unfortunately, none has been defined so far, either positionally or functionally. Such genetic variation is well documented in sheep (Gray, 1987; Stear and Murray, 1994), both when responses of different breeds are compared and when individuals within a breed are compared.

The challenge for animal breeders is to determine the best methods of using this variation to minimise infection and the effects of infection. The best methods for exploiting the genetic variation will depend on the traits which the breeder wishes to improve and on the relative importance of variation among and within breeds for these traits. The justification for including nematode resistance in a breeding programme is threefold.

- Nematode infections can be a major constraint on productivity. Coop et al. (1985) estimated that lambs infected with *O. circumcincta* grew approximately one-third more slowly than uninfected contemporaries and only one-third of this loss was recovered by anthelmintic treatment (Coop et al., 1982). There is also some indication that animals infected with nematodes may have carcasses of poorer quality (Coop et al., 1985). However, the genetic relationships between resistance and productivity are more contentious. Research performed in Australia on Merino sheep has shown essentially no relationship between resistance and productivity (Woolaston and Piper, 1996), while there are moderately unfavourable associations between resistance and productivity in dual-purpose sheep in New Zealand (Morris et al., 1997). The contrasting results could be a consequence of differences in the way the sheep are kept, the breeds of sheep studied or the species of parasites.
- There is increasing demand by consumers for meat products which are free of drug residues. Relatively resistant animals require less frequent treatment with anthelmintics (Morris et al., 1997).
- The third justification is that profitable and humane sheep farming in much of the world requires routine and regular anthelmintic treatment. Farming in these areas is threatened by the evolution of anthelmintic resistance in nematode populations. Therefore, breeding sheep which can survive and thrive without treatment is a desirable insurance against potential catastrophe.

Genetic variation between breed: The ability of animals to resist infections with parasites is genetically determined

and therefore variable between breeds or individuals of a given host species. While there is enormous variation in levels of resistance to disease, there are many cases where no breed has achieved complete resistance. It would be desirable to produce animals with even higher levels of resistance to disease, which would be able to thrive under the highest challenge in the absence of other disease control measures. There are good examples among small ruminants in which knowledge of the breed has had predictive value for resistance to parasitic infection (Courtney et al., 1985a, b; Mugambi et al., 1996a, b; Preston and Allonby, 1978, 1979; Wanyangu et al., 1997). In FAO's Domestic Animal Diversity Information System (DAD-IS), four goat breeds (Carpatina, Cashgora, Jamnapari, Katjang yei) and 13 sheep breeds (Churra Lebrijana, Criolla Mora, Criolla, Garut, Gulf Coast Native, Kumumawa, Madagascar, Malin, Morada Nova, Prianga, Rahmani, Solognot, Tsigai) were reported as having resistance or tolerance to a certain degree against parasitic diseases in general or against specific parasites (Gauly et al., 2010) in addition to Red Maasai sheep and small East African goats.

In general, resistant breeds come from areas with substantial exposure to natural infection and the superior resistance of such breeds may have arisen through natural selection. There have been many studies of the differences between breeds (Table 1). In USA, Florida Native was the first breed characterized as being more resistant to *H. contortus* infection in comparison with the Rambouillet breed (Radhakrishnan et al., 1972). Florida Native ewes are more resistant than exotic (St. Croix and Barbados Blackbelly), domestic (Finn-Dorset x Rambouillet) and exotic x domestic ewes (Courtney et al., 1985b). In North America, Stewart et al. (1987) followed FEC in grazing Rambouillet, Hamshire, Sharpsire, South down and Romney sheep from 6-20 months of age and found that Romney lambs were more resistant to *Ostertagia circumcinta*. St. Croix sheep were more resistant than Dorset and Rambouillet (Zajac et al., 1988). Bahirathan et al. (1996) and Miller et al. (1998) showed that Gulf coast native / Louisiana Native sheep are more resistant than Suffolk sheep. Scottish Blackface sheep are more resistant than Finn-Dorset sheep to *H. contortus* (Altaif and Dargie, 1978) and Red-Maasai sheep of Kenya are more resistant to breeds imported into Kenya (Preston and Allonby, 1978; Bain et al., 1993; Baker, 1995; Miller et al., 1995). Wanyangu et al. (1997) found that the Red Maasai sheep were more resistant to *H. contortus* than Dorper sheep. In France, Gruner et al. (1986) showed that Romnov sheep are more susceptible than Lacaune sheep to *O. circumcinta* infections.

Florida Native and St. Croix were reported to resistant for GIN (Zajac, 1995). In Indonesia Romjali et al., (1997) found that introduced St Croix ewes are more resistant than local Sumatra ewes to the parasites. The Indonesian Thin Tail sheep exhibit superior resistance to *H. contortus* (Subandriyo et al., 1996) as compared to Indonesian Fat Tail and Merino Sheep. A comparison between Barbados Black Belly sheep and INRA 401 composites demonstrated that Barbados Black Belly sheep have higher resistance to *H. contortus* and *T. colubriformis* than INRA-401 sheep (Gruner et al., 2003). Recently, breed resistance was assessed against nematode infection in Santa Ines, Ile de France and Suffolk male lambs in Brazil. It was found that the PCV and plasma protein values associated with high FEC and worm burdens in Suffolk and Ile de France lambs, while *H. contortus* and *O. columbianum* burdens were significantly lower in Santa Ines sheep. The relative resistance of Santa Ines young male sheep was superior to that of Ile de France and Suffolk sheep (Amarante et al., 2004). The breed difference in resistance to *H. contortus* was evaluated in crossbred Dorsets, Dorpers, straight-bred Katahdisan and Barbados Blackbelly x St Croix sheep in USA and found that Dorper sheep was not more resistant to parasites than Dorsets. The Katahdisan and Barbados Blackbelly x St Croix sheep were more resistant with lower FEC than Dorset or Dorper (Vanimisetti et al., 2004). A multi breed comparison showed that St. Croix, Gulf Coast Native, Katahdin and Suffolk were most resistant to most susceptible, respectively (Miller and Fernandez, 2005). In Africa Menz sheep were found more resistant than Horro sheep only under artificial challenge (Haile et al., 2002) and Red Maasai were more resistant than Dorper (Mugambi et al., 2005a, b). Santa line sheep in Brazil had higher resistance compared to other European breed (Bricarello et al., 2005; Costa et al., 2007).

Table 1. Resistance to parasites in different sheep breeds

Breed	Compared with	Type of infection	Results	Reference
Florida Native	Rambouillet, Hampshire	Artificial / Natural	Decreased FEC, Hb, worm count	Loggins et al (1965)
Florida Native	Rambouillet,	Artificial / Natural	Decreased FEC, Hb, PCV	Jilck and Bardley (1969)
Florida Native	Rambouillet	Artificial	Decreased FEC, worm count, PCV	Radhakrishnan et al (1972) Bardley et al (1973)
Navajo	Suffolk, Targhee, Rambouillet, Corriedale	Natural	Reduced number of worms	Knight et al (1973)
Malpura	Sonadi	Natural	Low susceptibility	Pachlag and Kumar (1974)
Targhee x Barbados	Targhee	Artificial	No difference in FEC, worm count, PCV, weight gain	Todd et al (1978)
Red Maasai	Merino, Corriedale, Hampshire	Artificial	Reduced FEC, worm count	Preston and Allonby (1978)
Red Maasai	Black-Head Somali, Merino, Dorper, Corriedale, Hampshire	Natural	Low FEC, worm count, higher survival	Preston and Allonby (1979)
Barbados black belly	Dorset and its crosses	Artificial / Natural	Decreased FEC, worm count	Yazwinski et al (1979)
Florida Native, St. Croix, Barbados	Rambouillet Finn Dorset X Rambouillet,	Artificial / Natural	Reduced or no PPR	Courtney et al (1984)
Florida Native, St. Croix	Finn Dorset x Rambouillet, Barbados Blackbelly	Artificial / Natural	Decreased FEC, no difference in worm count	Courtney et al (1985a,b)
Florida Native	Dorset x Rambouillet	Artificial / Natural	Decreased FEC, worm count	Zajac et al (1988)
Coimbatore	Merino, Rambouillet	Natural	Lower FEC, mortality, morbidity	Sanyal (1988)
Florida Native, St. Croix	Dorset x Rambouillet	Artificial	Decreased FEC, PCV, total protein	Zajac et al (1990)
Horro Arsi	Black-Head Somali Adal	NaturalLow	FEC, worm count, PCV, higher survival, body wt	Asegede (1990)
St. Croix	Dorset	Artificial / Natural	Decreased FEC, worm count, globular leucocytes	Gamble and Zajac (1992)
Munjal	Hisardale	Natural	Low susceptibility	Yadav et al. (1993)
Djallonke X Malin wool	Djallonke X Malin Dorset X Malin	Natural	Reduced FEC	Sani (1994)
Djallonke X Malin wool	Malin wool sheep	Natural	Reduced FEC	Pandey (1995)
St. Croix X Barbados	Sumatra X Javanese Fat Tail			
	Sumatra	Natural	Low FEC	Romjali (1995)
Blackbelly	St. Croix X Javanese Fat Tail			Romjali et al. (1997)
Red Maasai	Dorper	Artificial	Low FEC, PCV, higher survival,	Mugambi et al. (1996)
	Black-Head Somali, Dorper,	Natural		Mugambi et al. (1997)
	Romney,Dorper	Artificial / Natural	Low FEC, PCV	Wanyangu et al. (1997)
	Menz, Horro	Natural	Low FEC, PCV	Tembely et al. (1998)
Sumatra	Sumatra X St. Croix	Natural	Low FEC	Subandriyo et al. (1996)
Thai Native	Thia Native X Anglo-Nubian	Artificial	Low FEC, worm count	Pralomkarn et al, (1997)
Malpura	Avikalin, Bharat Merino	Natural	Low FEC	Swarnkar et al (1997)
Red Maasai	Dorper, Red Maasai X Dorper	Natural	Low FEC, PCV, higher survival, body wt	Baker et al. (1999)
Philippine Native	Anglo-Nubian X Boer X Saanen	Natural	Reduced FEC, PCV	Suba et al. (2000)
St. Croix	Katahdin, Rambouillet, Philippine Native	Natural	Reduced FEC, PCV	Suba et al. (2002)
Red Maasai	Dorper	Natural	Low FEC, PCV, higher survival	Baker et al. (2002)
Red Maasai	Menz, Horro	Natural	Low FEC, PCV, higher survival, body wt	Rege et al. (2002)
Menz	Horro	Artificial	Reduced FEC, worm count, PCV, higher body wt	Haile et al. (2002)
Red Maasai	Dorper, Red Maasai x Dorper	Natural	Reduced FEC, worm count PCV, higher body wt	Baker et al. (2003)
Sabi	Dorper	Natural	Reduced FEC, worm count, PCV, higher body wt	Matika et al. (2002)
Garole X Bannur, Deccani/ Bannur	Deccani, Bannur X Deccani	Artificial / Natural	Low FEC, PCV	Nimbkar et al. (2003b)
Garole	Malpura, Malpura X Garole	Natural	Low FEC	Singh et al. (2011)

These studies vary greatly in size, procedures by which breeds were sampled and the nature of the environment in which the animals were kept before and during testing. Nevertheless, for these three breeds at least, there is substantial evidence for relative resistance. Very few breeds have been thoroughly assessed for resistance, and there may be others of potential economic value with high levels of resistance which are worthy of investigation. Although the Merino breed may be relatively more susceptible to the diseases discussed here, most of the resistant breeds are hair or coarse wool breeds, and thus are unlikely to be attractive in cross-breeding programmes to increase disease resistance at the expense of decreasing wool production and quality.

From India, limited numbers of studies that are available have been predominantly on breed difference in susceptibility following natural exposure to disease. Since most of the studies are not aimed at assessing the genetic variation in resistance to diseases between and within breeds, they do not take in to account important factors such as whether the compared group were at the same location, whether they were reared in similar nutritional and managemental conditions. Pachlag and Kumar (1974) reported that Malpura sheep has low comparative disease susceptibility for haemonchosis compared to high susceptibility of Sonadi sheep in Rajasthan. Yadav et al. (1993) from Haryana observed that Munjal (Nali x Lohi) lambs had low susceptibility for *H. contortus* than Hisardale (Nali x Corriedale). Swarnkar et al. (1997) observed that susceptibility variation among different breeds of sheep with respect to FEC in weaner lambs naturally infected with *H. contortus* (predominantly). It was revealed that lambs of Malpura breed had the lowest FEC followed by Avikalin (50% exotic inheritance) and the highest FEC in Bharat Merino (75% exotic inheritance). Hooda et al. (1999) demonstrated distinct variations in FEC, haematological and biochemical parameters between responder and non-responder lambs exposed to *H. contortus.* It was also confirmed using purebred Garole rams grazed together with crossbred rams (Ghalsasi et al., 2009). Increase in Garole proportion led to a reduction in FEC and an increase in PCV (Nimbkar, 2006). An increment of 0.25 in Garole proportion was found to cause FEC after an artificial challenge to reduce by 1341 epg while another increment of 0.25 led to another decrease of 980 epg. Corresponding reductions in FEC after natural infection in ewes were 140 epg and additional 126 epg. These results indicated a polygenic basis of resistance to GIN in the Garole. The *FecB* gene did not have any influence on FEC or PCV (Nimbkar, 2006). Similarly, Singh et al. (2011) observed that Garole inheritance provides resistance against GINs to some extent, however *FecB* gene in Garole was not associated with intensity of infection.

Genetic variation within breed: Resistance to infection by gastrointestinal nematodes has moderate heritability in domestic sheep ranging from 0.13 (McEwan et al., 1992) to 0.53 (Baker et al., 1991) and resistant or susceptible lines have been selected in various countries (Dominik, 2005). Many studies have quantified within-breed heritability (Table 2), usually using FEC as the indicator of relative nematode resistance (Bishop and Morris, 2007). In almost all cases FEC, once appropriately transformed, is a moderately heritable trait and one which responds to selection. The heritability estimates reported in the literature for the FEC show a wide range of variation, from 0.0 to 0.55, as reviewed by Raadsma et al. (1997) depending on different factors, such as the population studied (e.g. sheep breed or animal age) or the nature of the parasite challenge (e.g. natural or artificial infection). Most of the estimations of genetic parameters for sheep resistance to GIN parasites have been carried out in sheep populations specialized for meat and/or wool production and managed under grazing-extensive production systems (Bishop et al., 2004; Morris et al., 2004), and the animals studied in most of the cases were lambs.

Albers et al. (1987) challenged lambs with *H. contortus* larvae and estimated the heritability of FEC after challenge as 0.34. In a study of the heritability estimates (h^2) of FEC in unselected 18 months old Merino rams following challenge with infective *H. contortus* larvae, Piper (1987) observed that based on the maximum FEC during four counts recorded 3-6 weeks after infection, the h^2 was estimated at 0.27±0.13 or 0.23±0.13 when the data were log transformed. In New Zealand there has also been a Romney line selected for high resilience and the heritability of the measure of resilience to nematodes was 0.14±0.03. More recently, Safari et al. (2005) have reviewed heritability estimates from many sources (published over the 1992-2003 years) and found a weighted average for transformed

FEC of 0.27±0.02, from 16 experiments. Heritabilities tend to be greater, on average, in experimental flocks than in industry data (Morris et al., 1995), partly because of the greater degree of control of management in experimental flocks, and perhaps because of higher degrees of challenge to the animals. Heritable resistance to *Nematodirus* species has also been reported (Morris et al., 2004), with heritability estimates of 0.15±0.03 in lambs of 4 months of age and 0.26±0.04 at 6 months of age, with genetic correlations of these with FEC data recorded at the same ages having a weighted average of 0.43. Studies on genetic correlation between resistance to *H. contortus* and *T. colubriformis* in INRA 410 sheep showed that the heritability of FEC of *H. contortus* ranged from 0.39 to 0.48 and genetic correlation between FEC after the first and second infection with the same or different species was near one. The similar heritability (0.47) was found with *T. colubriformis* and genetic correlation within and between species was also near to 1 (Gruner et al., 2004).

Table 2. Heritability of FEC following natural / artificial infection of GIN in sheep

Breed	Age	Infection type	h^2	Reference
Merino	Weaners	Artificial *T. colubriformis* after vaccination with irradiated *T. colubriformis*	0.41±0.09	Windon and Dineen,1984
Merino	Weaners	Artificial *H. contortus*	0.34±0.10	Albers et al., 1987
Merino	18 months	Artificial *H. contortus*	0.23±0.13	Piper, 1987
Merino	Weaners	Natural *H. contortus*	0.42±0.14	Cummins et al., 1991
Romney	Weaners	Natural *H. contortus*	0.53±0.15	Baker et al., 1991
Merino	Sheep	Artificial *H. contortus*	0.30 0.50	Woolaston et al., 1991
Romney	Weaners	Natural	0.34±0.09	Baker et al., 1991
Romney	Weaners	Natural *H. contortus*	0.13±0.07	McEwan et al., 1992
	Sheep		0.28	Nowosad et al., 1992
Ramanov	Sheep	Artificial mixed GIN infection	0.55	Gruner and Lantier, 1995
Polish long wool	Sheep	Artificial mixed GIN infection	0.28	Gruner and Lantier, 1995
B. Merino	Hogget	Natural *H. contortus*	0.42±0.27	Swarnkar et al., 1997
Red Maasai	Lambs		0.06	Baker et al., 1998
Polish long wool	Lambs	Natural *H. contortus* and *T. circumcinta*	0.22 - 0.33	Bouix et al., 1998
	Ewes		0.18 - 0.25	
Avikalin	Hogget	Natural *H. contortus*	0.35±0.18	Singh et al., 1999
Garole crosses	Sheep	Natural *H. contortus*	0.09 0.12	Nimbkar, 2006
Dorper	Lambs		0.35	Wolf et al., 2008
Malpura	Hogget	Natural *H. contortus*	0.010-0.246	Swarnkar et al., 2009
Avikalin	Hogget	Natural *H. contortus*	0.114-0.223	Singh et al., 2009
SardinianXLacaune	sheep	Natural	0.16	Sechi et al., 2009
Spanish Churra	Adult	Natural *T. circumcincta*	0.09-0.12	Gutierrez-Gil et al., 2010
Avikalin	Hogget	Natural *H. contortus*	0.149±0.096	Prince et al., 2010

In naturally infected population Sardinian x Lacaune backcross ewes and their daughters, Sechi et al. (2009) estimated heritability of 0.16. In a commercial population of adult Spanish Churra dairy ewes naturally infected with *Teladorsagia circumcincta*, Gutierrez-Gil et al. (2010) estimated heritabilities for the two FECs as 0.12 and 0.09. The genetic correlation between two FECS was 0.82. Woolaston et al. (1991) reported estimated heritabilities after artificial challenge with *H. contortus* of FEC in Merino ewes as 0.27, 0.22 and 0.31. A cube-root transformation was found to be effective in normalizing FEC data and reducing the range of with in selection line-birth year variance from 118-fold to 10-fold. Cummins et al. (1991) reported estimated heritability after naturally acquired *Ostertagia* infection of FEC in Merino sheep as 0.42.

The flock and sampling month were the external factors significantly influencing the heritability of FECs. This reflects the importance of the management given provided to the sheep, including management practices and protein content level in the diet (Martinez-Valladares et al., 2005), and the influence of the season as a factor directly controlling parasite development, especially in continental climates where hypobiosis phenomena are frequent (Almeria et al., 1996). Other factors, such as age and the physiological status of the ewe, significantly influenced several of the traits studied. Within-flock genetic investigations in research flocks and sire evaluation schemes allow public identity of sires with high estimated breeding values for disease resistance (Woolaston and Eady, 1995). The best way to use genetic variation within a breed is to set up a selective breeding scheme. Breeding for resistance to nematodes is no different, in principle, than breeding for any other trait. Breeders who already select sheep for improved production could incorporate nematode resistance into the selection programme. The success of a breeding programme will depend upon several factors, including the intensity of selection, the genetic make-up of the breeding animals, the accuracy of selection methods, the age of the parents and the size of the population.

Selection for resistance

The performance, adaptation and disease resistance of the vast majority of breeds in developing countries have not been systematically recorded. For any selection programme, it is essential that the superior animals should be identified accurately and economically from among the candidate breeding flock. Traits are of two types viz., predictor traits are those that can be measured in the absence of infection and infection traits associated with the course of infection. Following are the phenotypic indicator traits identified or used by several workers to assess the resistance GIN.

Predictor traits		Infection traits	
Traits	**Reference**	**Traits**	**Reference**
Whole blood lymphocyte	Riffkin and Yang, 1984	FEC	Mckenna, 1981
culture stimulation index	Cummins et al., 1991		
Ovine lymphocyte antigen type	Outteridge et al., 1988	Blood eosinophil count	Windon, 1991,Stear et al., 2002
Haemoglobin type	Agar et al., 1972	Resilience	Albers et al., 1987
	Coutney et al., 1985		Morris et al., 1995
Antibodies to larval antigen by ELISA	Morris et al., 1995	Antibodies to larval antigen by ELISA	Morris et al., 1995
		Faecal antigen test	Karlsson et al., 1991
		Immune responsiveness	Gill et al., 1993
		Parasite specific IgA activity	Strain et al., 2002
		Pepsinogen concentration	Stear et al., 1999
		Plasma albumin concentration	Stear et al., 2000
		Fructosamine concentration	Stear et al., 2001

There are several direct and indirect ways for selection of resistance. Direct traits involve enumeration of the total number of parasites carried by the animals and require slaughter and collection of worm from the GI tract. Thus, an indirect measurement of resistance is required for the selection of breeding stocks. Indirect traits can either be associated with parasite infection (infection traits) or are independent of infection and predict the response to infection when challenged (predictor traits). Direct selection can be performed by three different approaches.

i. Animals may be observed in a given production system or environment for lack of clinical expression of a disease. In this approach, it is assumed that the disease pathogen is constantly present. However, the expression of disease resistance is questionable. Animals with clinical expression of the disease may be identified with relative accuracy but not all healthy animals may be exposed to the pathogen or challenged equally. Also, disease exposure in natural environments is subject to temporal and spatial clustering of

disease incidence. Diseases often occur in clusters of time (years, seasons, production cycles, etc.) and space (flock, pasture, farm, region, etc.). In years when the disease incidence is high, there can be an increase in the accuracy of identifying animals with a high probability of being disease resistant but in years of low incidence the accuracy will be diminished (Snowder et al., 2005).

ii. By uniformly challenge of all breeding stock with infection. It can be costly depending upon the pathogen's virulence and clinical expression of the disease but is a reliable measure of disease resistance. This may require isolation of the population to prevent transmission to non-breeding stock.

iii. By challenging the relatives or clones of the breeding stock, especially if the disease has a high mortality rate. It is also a reliable method of determining genetic resistance.

Indirect selection can be accomplished by measuring the continuously variable physiological (pathogen reproductive rates, pathogen byproducts) or immunological traits (indicator traits) or by polymorphism of a single gene or small group of linked genes (molecular genetic markers). For effective selection, indicator traits must be heritable, highly genetically correlated with resistance to the disease or diseases of interest, accurate to measure and affordable. Interactions between the genetics of the animal and the environment commonly exist. If the genetic by environmental interaction is significant, animals selected for improved disease resistance in one environment may be more susceptible to the same disease in a different environment. Therefore, selection programs may have to be environment specific with the selection environment matching the commercial production environment.

Under ideal circumstances, the identification of genetically superior animals would be possible without the need for direct challenge. Understanding the mechanisms responsible for disease resistance or the genes responsible for resistance offers this potential strategy. Although the major diseases which adversely affect production are generally accepted to be under polygenic control, this does not exclude the possibility that genes of relatively large effect could operate. In a polygenic model with moderate heritabilities, many factors (most of which are non-genetic) contribute to resistance. The problem is to separate the genetic from the non-genetic factors. This requires specialized experimental resources. Most of the current understanding of genetic mechanisms of disease resistance in sheep comes from comparisons of animals in lines selected for and against a particular disease. Even then gene effects cannot always be distinguished from other effects or single gene effects distinguished from other polygenic effects. Most experiments designed for detailed investigation of disease resistance lack sufficient power to detect the effects of interest (Raadsma et al., 1988).

Faecal egg count: FEC is the trait most widely used to monitor nematode resistance. It is an indirect in one sense, because it indirectly measure worm number, which were used to define resistance. In other sense, however, reduced FEC has some value of its own as a breeding objective, as it provides a direct measure of pasture contamination (Singh and Swarnkar, 2010). FEC has been established as a good indicator of strongyle worm burden with a correlation coefficient of 0.74 between individual egg counts and worm counts in sheep up to 12 months of age (Mckenna, 1981). This correlation was lower (0.23) in adult sheep, presumably due to suppression of egg production as a result of a strong immune response. Bisset *et al*. (1991) observed high correlation between FEC and total worm burden in both resistant (0.83) and susceptible (0.75) groups of Romney sire progenies. The correlation between FEC and total worm burden was 0.836 in Avivastra sheep (Rambouillet X Chokla) artificially infected with *H. contortus* (Swarnkar, 2000). Somewhat surprisingly, the heritabilities of FECs following deliberate infection are similar to those of egg counts following natural infection. The heritability is the ratio of the additive genetic variation to the total variation and indicates the likely response to selection. The additive genetic variation represents the sum of the average effects of all the relevant genes. Therefore, there is little justification for using deliberate infections, except in situations where the parasite challenge varies widely from year to year due to changes in weather conditions. Three factors which do

influence the heritability of FECs are the age and exposure of the animals studied, the precision of the egg count and the number of samples examined. Resistance to nematode infection is probably an acquired and not an innate response. Thus, for natural infection, the heritability increases from essentially zero at one and two months of age to 0.33 at six months of age (Bishop et al., 1996a; Stear et al., 1997). This is due to a combination of age and exposure, but the precise contribution of each component is unknown. In practical terms, host resistance to endoparasitism is now known as a heritable trait and is used in industry programmes (WormFEC in New Zealand and Nemesis in Australia). Genetic progress in FEC is being made in flocks where selection is applied, with heritabilities of ~0.2 (depending on FEC-sample timing) in New Zealand, and 0.22 in Australia (Eady, 2009). The selection programme for establishing resistant (low FEC) and susceptible (high FEC) lines have been developed in New Zealand, Australia and India. Breeding values for FEC are generally available to ram breeders to allow them to breed for increased worm resistance in Australia (www.sheepgenetics.org.au) and in New Zealand (www.sil.co.nz). Prediction and publication of such breeding values have resulted in significant genetic changes in industry flocks both in Australia and New Zealand (Amer, 2009).

As FEC meet many of the above requirements and is reasonably practical indirect, it is the only measurement to date that has been used extensively to measure the parasite resistance of sheep. It's major disadvantage is that it is a disease trait and the animal must become parasitized to obtain a resistance measurement. Repeatability estimate (r) is the correlation between repeated measures on the same animal or the proportion of the variance of a measure on a group of animals. Repeatability analysis allows the separation of variation in FEC due to temporary (environmental) and permanent (genetic and environmental) effects. The repeatability of a trait sets an upper limit of its possible h^2.

Repeatability within an infection cycle: Within an infection cycle, the repeatability of two FECs made a week apart, 4-5 weeks into the infection is high. Repeatability estimates were 0.6-0.7 for a single artificial infection of *H. contortus* (Woolaston et al., 1991) and 0.69 for artificial infection of *T. colubriformis* after vaccination with irradiated *T. colubriformis*. Over an extended infection, r of fortnightly FECs was also found to be high (0.6) for an artificial trickle infection with *H. contortus* (Barger and Das, 1987), persisting for 13 weeks.

Repeatability between infection cycles: The r between artificial infections with *H. contortus* was found to be 0.3 (Barger and Das, 1987). In New Zealand flocks, the r of FEC on two consecutive natural infection cycles ranged from 0.4 to 0.5 (Baker et al., 1991) and for Australia flocks, it ranged from 0.11 to 0.48 with a mean of 0.27 (Cummins et al., 1991). The time for the completion of the two-infection cycle in each country was approximately 3 months. When sheep are exposed to parasite infection from birth with no drenching, it appear that r of FEC from month to month is low (0.03-0.04) and variable (Karlsson et al., 1991). This may be due to animals being at different stages of resistance development, unadjusted by anthelmintic treatment which can itself cause a loss of natural resistance (Barger, 1988). In India, Swarnkar et al. (2000) estimated r of log transformed FEC to the tune of 0.49 between two cycles of natural *H. contortus* infection and found that log transformed worm count has significant positive (0.836) correlation with transformed FEC. The multiple regression analysis showed that both FEC and PCV together accounted for 73% of the variation in the worm burden, while FEC, Hb, PCV, TEC and body weight traits together accounted for 86% of the observed variation in the worm burden. Steer et al. (1996) reported r of the FEC and TEC to the tune of 0.85 and 0.78, respectively while Barger and Das (1987) observed r of 0.29 for FEC and 0.26 for PCV in sheep experimentally infected with *H. contortus*. The correlation coefficient between FEC and worm count in sheep were positive and reported to the tune of 0.74 (McKenna, 1981), 0.75-0.83 (Bisset et al., 1991) and 0.83 (Swarnkar et al., 2000). To gain the highest return from the investment of measures in a breeding programme for parasite resistance, it would be of greater benefit to measure FEC over two consecutive infection cycles, rather than with in the one cycle or over a longer interval.

At CSWRI, Avikanagar, from the year 2004, research was initiated within breed selection of resistant and / or susceptible sheep with the aim to create divergent lines in native (Malpura) and cross bred (Avikalin) sheep. By using FEC as phenotypic marker and the protocol for selection (Fig. 1) two divergent lines (resistant "R-line" and susceptible "S-line") were created. Since then selected animals are being monitored for intensity of infection and production performance.

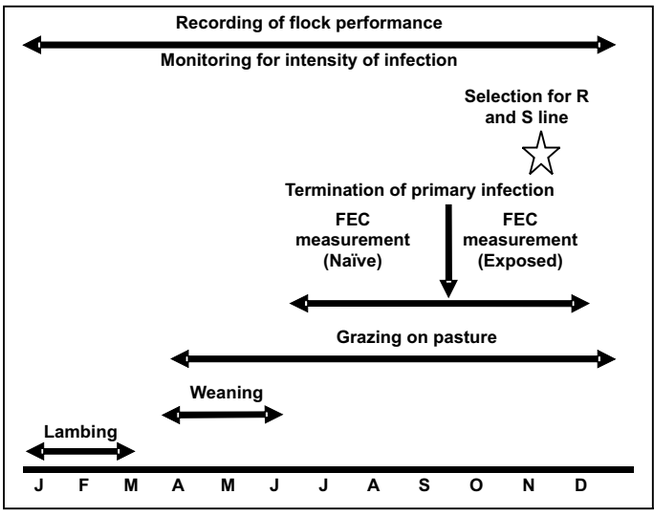

Fig. 1. Study protocol for selection of sheep for divergent lines

The analysis of data for intensity of strongyle infection in divergent lines over the years exhibited that in both the breeds animals of R-line had lower monthly FECs compared to their counterparts in S-line (Fig. 2 and 3). The animals of S-line required strategic as well as tactical anthelmintic intervention while animals of R-line were maintained without any anthelmintic intervention.

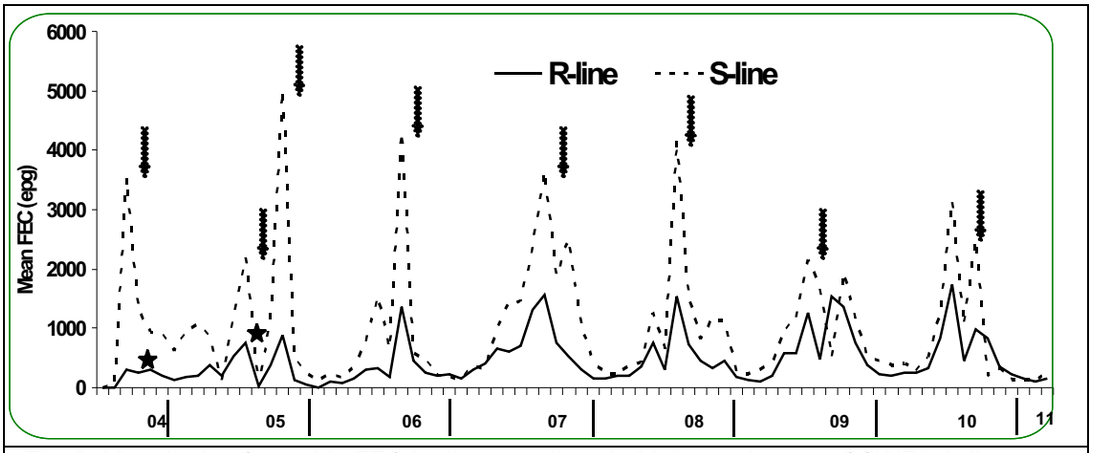

Fig. 2. Magnitude of monthly FEC in divergent lines in Malpura sheep at CSWRI, Avikanagar (From July, 2004 to March, 2011; Anthelmintic intervention in susceptible (arrow) and resistant (star) sheep)

The magnitude of monthly body weight changes in adult animals revealed non significant variation in both R and S-line. In comparison to initial body weight, at the end of year variation in body weight ranged from + 0.62% (R-line) to + 2.89% (S-line) in Malpura breed and from + 4.25 % (R-line) to 0.57% (S-line) in Avikalin breed. The mean annual GFY for animals of R and S-line did not differ significantly from each other in both the breeds. It ranged from 1.021±0.049 kg (R-line) to 1.038±0.049 kg (S-line) in Malpura breed and from 1.534±0.074 kg (S-line) to 1.571±0.073 kg (R-line) in Avikalin breed. The overall annual tupping and annual lambing on tupped basis was 91.1% and 84.3%, respectively in R-line while 93.2% and 85.4% in S-line revealing non-significant variation among both the line. The annual % mortality ranged from 5.59±1.31 (R-line) to 7.21±2.73 (S-line) and from 6.74±2.70 (S-line) to 7.57±2.96 (R-line) in Malpura and Avikalin breed, respectively. Like-wise % culling / sale / transfer per annum varied from 5.42±2.29 (R-line) to 7.37±2.99 (S-line) in Malpura and from 6.66±2.62 (R-line) to 7.82±2.61 (S-line) in Avikalin flock.

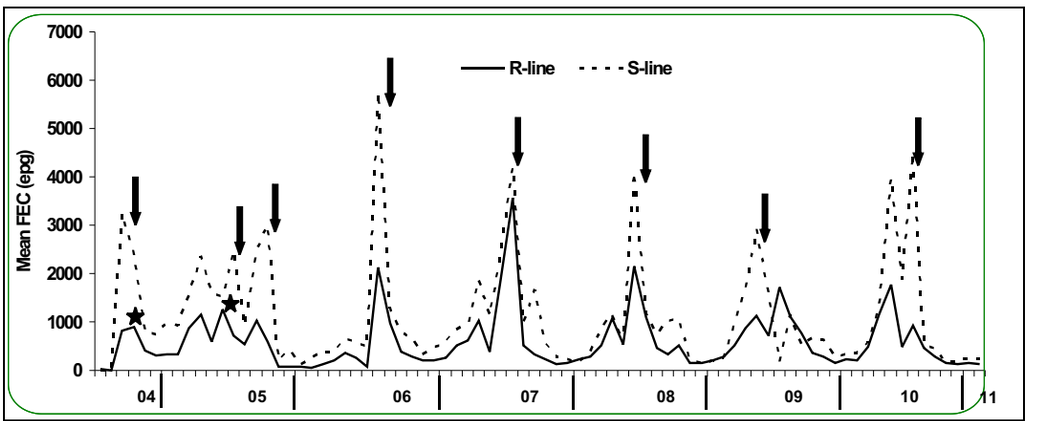

Fig. 3. Magnitude of monthly FEC in divergent lines in Avikalin sheep at CSWRI, Avikanagar (From July, 2004 to March, 2011; Anthelmintic intervention in susceptible (arrow) and resistant (star) sheep)

The evaluation of progenies born from matting with selected sires exhibited that in both the breeds progenies having inheritance of R-line possess comparatively lower FEC than those having inheritance of S-line. The findings are suggestive that strategy of selection and breeding of resistant animals will able to develop a flock that had lower FEC during wormy season and led to reduced pasture contamination. The growth performance revealed that in both the breeds, progenies born from S-sire had slightly higher body weight at different stages of growth compared to those sired by R-sire except at birth in Malpura breed. However, in Avikalin breed there was marginally higher growth rate in lambs from R-sire up to 6 month of age.

Blood eosiniphil count: Windon (1991) observed that sheep selected for high responsiveness to vaccination against *T. colubriformis* showed high responder and had increased blood eosinophil counts. The difference between the high and low responding lines being significant only after infection, when the immune response was having in greatest impact on the parasite burden. The relatively low level of circulating eosinophils in the low responder during parasite infection suggest that eosinophil response was a more a measure of immune mediated response rather than level of burden (Dawkins et al. 1989). The use of eosinophil count as an indirect trait for FEC has been 40% efficient as using FEC trait itself. Eady (1995) stated that based on the available estimates for genetic parameters for blood eosinophil counts, it appear that this trait alone would not be a useful indicator of parasite resistance.

Packed cell volume: PCV following infection with *H. contortus* is a valuable marker for blood-sucking parasites, but has no value for infection with other nematodes (Woolaston and Piper, 1996).

Immunoglobins A (IgA): The concentration of antiparasite antibody is routinely used in New Zealand to assist in the identification of resistant sheep (Douch et al., 1995). Studies performed in Scotland of lambs infected with *0. circumcincta* suggest that IgA is the important antibody and fourth-stage larvae are the important stage of the parasite life-cycle (Sinski et al., 1995; Stear et al., 1995). By anti-parasite antibody studies, Green *et al*. (1999) have shown in New Zealand that mixed-species challenge during the genetic selection process has led to host resistance to various individual parasite species. In Australia, Eady (2009) has shown for the *Haemonchus* selection lines that there is effective cross-resistance to different parasite species.

Whole blood lymphocyte culture: Lymphocyte responsiveness to nematodes develops early in the life of sheep in the absence of the parasites, possibly as a genetically controlled response to heterophile antigens. Pre-infection lymphocyte stimulation index (SI) level gave the best single prediction of the potential of sheep to resist *H. contortus* infection. Cummins et al. (1991) estimated h^2 of the lymphocyte SI at moderate level (0.29±0.13) but not as heritable as FEC (0.42±0.14). The efficiency of the SI varies with the effect of a nematode challenge at the time of testing. Ideally the sheep should be worm free and under minimal challenge from pasture for the base level of intrinsic response to be measured accurately. In the high resistance line selected for low FEC and high SI, FEC made the greatest contribution to resistance and the flock has continued to be selected for FEC alone.

Ovine lymphocyte antigen (OLA): These are glycoproteins and are a sub group of the major histocompatibility complex (MHC). OLA type can be determined by serological testing and it is polymorphic, controlled by a number of loci. Outteridge et al. (1985) found different incidence of OLA type between the high and low responder lines of sheep selected for and against responsiveness to vaccination against *T. colubriformis,* the high responder having a greater incidence of SY-1type. Windon et al. (1987) observed significant divergence in FEC of the two lines, which may indicate an association between FEC and OLA type. A consistent relationship between FEC and OLA type has not been demonstrated with in selection lines. Luffan et al. (1986) reported the possibility of OLA markers in relation to genetic resistance to *H. contortus.*

Haemoglobin type: Initial studies with sheep of different haemoglobin type suggested that sheep of haemoglobin type A may harbor fewer worms than sheep with haemoglobin type AB or B following *H. contortus* infection (Evans et al., 1963; Allonby and Urquart, 1976). Using haemoglobin type as a selection criterion for parasite resistance is likely to be successful due to the inconsistent effect of haemoglobin on resistance. However, recent studies were unable to find any difference in parasite resistance between Hb genotypes (Kassai et al., 1990).

ELISA: Morris et al. (1995) reported that resistant sheep could be identified by their antibody response to parasites, measured by ELISA, which include both *T. colubriformis* and *H. contortus*. In weaners, the h^2 for *T. colubriformis* antibody response was 0.27±0.08 and the genetic correlation between the first FEC measurement and ELISA was -0.56±0.18. The antibody response in the absence of infection is indicative of parasite resistance, which has the added benefit over FEC of reducing the impact of the disease on production during the assessment of resistance studies.

Body weight gain / growth rate is another marker for disease resistance. The genetic correlation between growth rate and egg count is so strong (-0.8) that selecting sheep for growth rate automatically selects for decreased FECs (Bishop et al., 1996a).

Such direct approaches of phenotyping the animals for disease resistance would take place in a highly controlled and isolated environment. There is only one direct way to measure nematode burdens, but this method could not be employed as a selection criterion.

Immune responses: These are certainly implicated in the genetic resistance of sheep to worm infections (Gray and Gill, 1993). Lines of sheep successfully selected on the basis of response to vaccination against T. *colubriformis*

(Windon, 1991) provide further evidence that there is genetic variation in the ability of the host to respond to vaccination as well as to infection. In lines of selected sheep, immune or inflammatory defence mechanisms have been shown to be associated with resistance to the parasite (Cloditz et al., 1996; Gill, 1991; O'Meara et al., 1992). A characteristic of the immune response to nematode infection is an accumulation of mucosal mast cells (MMC) and eosinophils in the gastrointestinal tract (Rothwell, 1989). The MMC accumulation is antigen-dependent and is driven by the T lymphocytes, and these MMCs undergo changes to globule leucocytes during infection (Huntley et al., 1984). Genetically resistant sheep have high numbers of globule leucocytes relative to non-resistant counterparts (Presson et al., 1988). As the immune response to helminths becomes better understood, the possibility of using these traits as indicators of resistance will increase.

Genetic markers

Maps densely populated with genetic markers are potentially a vary important tool in animal breeding as they allow detection of genes affecting important production traits and hence the possibility of basing selection in future breeding programme on genotype rather than phenotype. Identifying the genes which code for resistance to nematode infection could increase the accuracy of identifying genetically superior animals and could enable the ranking of animals with different histories of exposure to infection. Searches for associations between genetic markers and disease resistance have focused mainly on resistance to nematodes and footrot. Such studies also require specialized genetic resources to follow the segregation between marker and resistance. Although possible associations between resistance and haemoglobin type, gene(s) within the major histocompatibility complex and anonymous DNA markers have been reported, there is a lack of consistency in these relationships. It is still unknown that whether resistance is conferred by a small number of genes or a larger number of genes. Two strategies can be used to identify genes influencing resistance. The first strategies does not require an existing genome map and use the analysis of candidate genes (expressed genes) that may be expected to play a role in regulating resistance (i.e. gene encoding immunoglobulins, MHC antigens, T-cell receptor molecule etc.). The second relies upon the linkage maps and genome wide analysis for quantitative trait loci (QTL) detection. This method is based upon the use of polymorphic DNA markers to tag specific genes or regions of the genome carrying resistant gene. The application of knowledge from mapped microsatellite markers offers a powerful tool to design linkage experiments which will point to the chromosomal location of genes with a major additive effect on disease resistance.

Haemoglobin type: The first genetic marker used in the selection of resistance was haemoglobin type. Sheep have two alleles (A and B) for haemoglobin and animals with haemoglobin type AA were more resistant than AB which were more resistant than BB with *H. contortus* infection (Allonby and Urquhart, 1976; Altaif and Dargie, 1978). However, these haemoglobin types could not be effective as markers for resistance to *H. contortus.*

Major histocompatibility complex (MHC): The MHC gene family can be shown to contain two different clusters of genes (class I and II) that code for cell surface glycoproteins involved in antigen presentation to T lymphocytes. Three loci appear to be associated with parasite resistance are interferon gamma gene (IFN-γ) on chromosome 3 (Coltman et al., 2001); the major histocompatibility complex (MHC) on chromosome 20 (Paterson, 1998; Paterson et al., 1998); and the adenosine deaminase gene (ADA) on chromosome 13 (Gulland et al., 1993). Research in several species of hosts has demonstrated that genes in or around the MHC are associated with resistance to nematode infection (Outteridge, et al., 1996; Schwaiger, et al., 1995; Stear et al., 1988, 1989, 1996; Van Haeringen *et al*. 1999; Wakelin, 1988). There has also been considerable interest in the association between MHC genes and disease resistance in domestic animals (Lewin, 1989; Nonnecke and Harp, 1989; Ostergards et al., 1989; van Der Zijpp and Egberts, 1989). OLA in sheep (Spooner et al., 1985), CLA in goats (Nesse and Larsen, 1987) and ELA in horses (Bailey et al. 1988) have been documented.

The MHC is a multigene family controlling immunological self/non-self recognition. Amongst them are the genes that encode the cell surface glycoproteins that present peptides of foreign and self proteins to T cells, thereby controlling all specific immune responses, both cell- and antibody-mediated (Klein, 1986). Compared to other domesticated species, sheep MHC is poorly characterized and have distinct class I and II regions. Sheep MHC class II gene has been shown to be highly polymorphic and believed to play a major role in immune defense against macro parasites (Klein et al., 1993, Weir and Stewart, 1993). Grain et al. (1993) reported the restriction fragment length polymorphism (RFLP) of DQB and DRB class II genes of ovine MHC using probes, but no genetic association with parasite resistance could be established. The SSCP (single strand conformational polymorphism) technique demonstrated that the segregation of alleles was found with both of intronic microsatellites and exon 2 variable regions (Outteridge et al., 1996). Nineteen DRB1 alleles were identified within the intron between exon 2 and 3 and suggests that the MHC complex plays an important role in the development of resistance to *O. circumcincta (*Schwaiger et al., 1995). Paterson et al. (1998) analyzed MHC variation in Soya sheep using five microsatellite markers. Markers OLADRB and OLADRBps are located within MHC class II expressed and non-expressed genes, respectively (Blattman and Beh, 1992), while OMHC1 is located within the MHC class I region (Groth and Wetherall, 1994) and BM1815 and BM1818 used as flanking markers. They found that OLA-DRB locus is strongly associated with juvenile survival and alleles significantly associated with parasite resistance in lambs and yearling. Interestingly, the OLADRB 257 allele was significantly associated with both decreased parasite resistance and decrease survival in lambs, while the OLA-DRB 263 allele is associated both increased parasite resistance and increased survival in yearlings. Exon 2 of the *Ovar-DRB*1 gene codes for part of the MHC class II antigen binding cleft and over 80 alleles have been identified at this locus in sheep (Sayers et al., 2005). Thus, the parasites are likely to play a major role in the maintenance of MHC diversity in the population.

The influence of MHC genes can be difficult to estimate but may be quite strong (Kennedy et al., 1992). For example, six-month-old Scottish Blackface lambs with the G2 allele of the *DRB1* locus have egg counts over 50 times lower than lambs without this allele. This locus alone accounts for two-fifths of the genetic variation in faecal egg counts. In another study, Schwaiger et al. (1995) identified an ovine MHC class II antigen which associated with 98% lower egg count in Scottish Blackface sheep naturally infested with *O. circumcincta* in Scotland. A subsequent study showed that DRB1 class II antigen was associated with 10-fold reduction in faecal egg count. Special attention is paid to the MHC class II molecules that induce the immune response in case of extracellular infection.

A number of indigenous “unimproved” breeds of sheep appeared to be significantly resistant or tolerant to parasites as compared with “improved” breeds. Studies on primitive Heatherheaded sheep, showed a significant association between microsatellite polymorphism in *DRB1* gene and FEC (Charon et al., 2002). The investigated gene fragment was found highly polymorphic and a total of 23 alleles were identified. Alleles 482 bp and 530 bp showed significant association with resistance to GINs, while 568 bp allele was found related to susceptibility to parasites. Charon (2004) studied the RFLP in exon 2 of *DRB1* gene in relation to nematode parasite resistance/susceptibility. Amplified exon 2 was digested with *Bsu*RI, *Rsa*I and *Bst*YI restriction enzymes and found 10 RFLP patterns using *Bsu*RI, eight using *Rsa*I, and only two patterns using *Bst*YI enzyme. Two restriction patterns *Bsu*RI and *Rsa*I were newly identified. Sequence analysis of DNA samples confirmed new sequences of the exon 2 of *DRB1* gene, registered in the GenBank, accession numbers AY230000 and AY248695. The *DYA* gene (belonging to the class IIb sub-region of MHC) closely linked to the microsatellite *DYMS1* is a possible candidate gene for resistance to *H. contortus* in Rhönschaf sheep of Germany (Janssen et al., 2002). MHC- associated locus, OLA-SY1 (SYla + SYlb) was found associated with increased responsiveness to vaccination (decreased FEC) following *T. colubriformis* challenge parasite challenge in Australian Merino (Outteridge et al., 1985, 1988) and Romney sheep (Douch and Outteridge, 1989), while SY6 was found to associated with increased FEC.

The polymorphism of the *Ovar-DRB*1 gene plays an important role in resistance to nematode infection in the Suffolk breed (Sayers et al., 2005). Suffolk sheep carrying the DRB1*1101 (previously referred to as-DRB1*0203 or G2) allele have been reported to show increased resistance to natural *T. circumcincta* infection compared to non-carriers. Resistance conferred by DRB1*1101 is acquired rather than innate, depends on worm expulsion rather than fecundity and is dependent on mucosal mast cell proliferation, platelet activation, and IgA and IgE antibody responses (Hassan et al., 2011). Ovine resistance to *T. circumcincta* develops over time (Smith et al. 1984; Emery, 1996) and is dependent on the activation and development of a Th2 immune response, characterised by the production of anti-inflammatory cytokines such as interleukin (IL)-4, IL-5, IL-9, IL-13 (Craig et al., 2007; Ingham et al., 2008; Keane et al, 2006), recruitment of mast cells and eosinophils in the mucosa (Huntley et al., 1995; Kemp et al., 2009; Miller, 1996), production of IgA (Smith, 2007; Halliday et al., 2007, 2010; Stear et al., 2004) and IgE (Sayers et al., 2008) It is known that proper antigen presentation is essential to the development of a successful immune response (Cigel et al., 2003). Therefore, owing to the importance of the MHC molecules in antigen recognition and presentation (Bernatchez et al., 2003; Dukkipati et al., 2006; Piertney and Oliver, 2006), and the fact that an allele of the MHC-DRB1 locus has been associated with increased nematode resistance in sheep (Stear et al. 1996; Sayers et al., 2008; Schwaiger et al., 1995), it is plausible that MHC genes play a key role in determining the outcome of the immune response and nematode infection in general. Particularly, polymorphisms within the DRB1 locus that encode the b1 domain of the MHC-II molecule which constitutes the peptide binding region (Brown et al., 1993) are likely to play a pivotal role in disease outcome.

At CSWRI, Avikanagar efforts are in progress to study the polymorphism of MHC *Ovar-DRB1* gene in resistant (R) and susceptible (S) lines developed against *H. contortus.* The *Ovar-DRB1* of 296 bp was amplified through nested PCR. In Malpura breed, *SacI* produced three genotypes with predominance of AB genotypes in R line compared to AA genotype in S line. RE pattern with *Hin1I* exhibited non significant variation among both the line and genotypic frequencies remained almost same. There was higher frequency of AB genotype in R line compared to AA genotype in S line with *NciI* enzyme. No significant variation in genotypic and allelic frequency was noticed among r and S line with *SacII* enzyme and population was skewed towards homozygocity. *BstN1* RE pattern exhibited no significant difference between the R and S lines. The RE produced three RE patterns having highest value of AB pattern in both the lines. In Avikalin breed there was no significant difference in the genotype produced by *NciI, Hin1I, SacI, SacII* and *BstN1* enzymes between R and S lines. So far study revealed that Ovar-DRB1 is highly polymorphic in both the lines R and S lines in Malpura and Avikalin. Although no association could be traced out between the frequencies of RE pattern and the lines in either of the breeds and study on more number of samples is needed to reach some conclusions.

A corollary of the strong effect of these MHC genes is that additional genes at other loci may be difficult to detect. The additional genes are unlikely to explain as much variation as the MHC and their influence will be weaker. Many genes which influence immune responses to nematodes are likely to be very polymorphic. Any serious search for genes in outbred populations will require large numbers of animals to ensure that rare but potentially important alleles are included in the study population. The problem is even more acute with linkage analyses or genome-wide screens, because these analyses need a significant number of sires to allow for polymorphism and a large number of offspring from each sire to detect relatively small effects. Care must also be taken with these studies to take into account sire or founder effects which can easily distort MHC association findings when inbred groups of animals are studied.

Quantitative trait loci (QTL): Disease resistance and adaptation traits are generally very difficult to record and often have low heritability. Specifically, effective direct selection for disease resistance requires that:

- Animals are exposed to disease
- The degree of challenge received by each animal can be recorded
- The response to disease challenge can be accurately recorded
- The disease exposure is ethically acceptable
- Animals are capable of breeding efficiently after disease exposure.

The concept of detecting and utilising genes that control disease resistance was first introduced in the 1970s. The development of genetic markers for parasite resistance in domestic sheep has been received major attention in the last decade. Till date, several studies were made on the use of molecular genetic markers to detect the presence of genetic loci controlling quantitative genetic variation (i.e. quantitative trait loci, QTL). QTL studies, association analysis and the search for selection signatures allow identification of genomic regions that show polymorphisms associated with tolerance or susceptibility. QTL mapping can help to dissect the complexity of parasite resistance by identifying candidate genomic regions affecting the trait variation. In both livestock and model species, many hundreds of QTL have now been mapped. QTL are now being used in genetic improvement programmes for several species in the developed world (Gibson and Bishop, 2005). Several studies have identified QTL for host resistance in sheep (Dominik, 2005).

For traits that are easy to record and have high heritability, conventional phenotype-based selection methods will produce good genetic progress and use of QTL is generally expected to yield only small increases in genetic progress or small reductions in costs. Use of QTL is predicted to be most beneficial for traits that have low heritability or are difficult, expensive or impossible to record. In this regard, use of QTL could be expected to be particularly beneficial in the low- to medium-input systems of the developing world, where the disease resistance and adaptation of livestock are critically important for the sustainable livelihoods of poor farmers. Different QTL will have different impacts on the overall transmission of infection and those QTL that reduce the disease impact by considerably reducing susceptibility to infection are generally the most appropriate to use (Nath et al., 2004). Bishop and Morris (2007) and Stear et al. (2009) reviewed the resistance to nematodes in sheep and listed main QTL identified depending on the breed, the parasite species, type of infection and the environment. The most interesting QTL lies in OAR 3, in INF-γ region, in OAR 20 and in the CMH region.

Research conducted at Sydney University and the University of New England in Australia, has involved segregation analysis to investigate evidences for the segregation of a major gene for nematode resistance. A QTL for resistance to *H. contortus* was segregating within a flock of Indonesian Thin Tail x Merino crosses based on FEC after primary artificial challenge. However, the Golden Ram project also shown evidence for a QTL in the experimental flock. Microsatellites can be applied to assess neutral genetic variation within or among individuals and populations. They provide markers useful for deriving pedigree relationships and can act as markers for regions of the genome containing QTL or candidate genes of importance. Three questions required to be addressed using microsatellite DNA information are i) is there heritable variation for resistance, ii) is resistance compromised by inbreeding and iii) what genes are associated with resistance?

Three loci (TCRG4, ADCYAP1 and MMP9) in genes with disease-related functions were associated with lungworm abundance. TCRG4 (T-cell receptor) has an immune system function involving recognition of foreign antigens. Diversity at this locus could allow the recognition of a more diverse range of parasites. The ADCYAP1 gene (adenylate cyclase-activating polypeptide) is involved in the regulation of cytokine production including interleukin 6 that activates the production of T-helper cell 2 (Th2) cytokines involved in defence against helminths and other extracellular parasites (Mosmann and Sad, 1996). Furthermore, ADCYAP1 was recently found to be associated with

nematode parasite infection in domestic sheep (Crawford et al., 2006). Average individual heterozygosity at the microsatellite loci was negatively associated with FEC indicating that relatively inbred sheep are more susceptible to parasitism by nematodes. Coltman et al. (1999) observed that low heterozygosity is associated with increased nematode parasitism and lower survival in feral Soay sheep. Similarly, Luikart et al. (2008) also found that reduced genetic variation can increase host susceptibility to parasites in wild bighorn sheep.

Marker Assisted Selection (MAS) offers opportunities to make faster genetic gain for traits such as disease resistance that is difficult to measure (McEwan, 2009). The first commercial genetic marker (WormSTAR™) for worm resistance was recently released and marketed by Catapult Genetics of Pfizer Animal Health in New Zealand (www.catapultsystems.co.nz). The WormSTAR™ marker explains approximately 2.3-3.6% of the genetic variation for the FEC traits, 4.8-5.5% of the live weight traits, 3.7% for the wool traits and 6.2% for lean weight (McEwan et al., 2008).

In New Zealand, divergent sheep selection lines resistant and susceptible to nematode parasites were used to find the respective QTLs (Diez-Tascon et al., 2002). The QTL for resistance was localized in chromosome 3 and mapped to about a 5 cM region. The gene located to this region codes for the interferon gamma (IFN-γ) and is considered a putative candidate gene for resistance to nematode parasites. In Australia the study to identify QTL for resistance to T. colubriformis in Merino sheep families was undertaken by Beh et al. (2002) who showed the respective QTL on chromosome 6 (LOD score = 4.2). In Germany the study on QTL for resistance to parasites was carried out on the Rhönschaf sheep (Janssen et al., 2002). Statistical analysis showed significant association between parameters of resistance (faecal egg count) and the markers *OarCp73*, *DYMS1* and *BM1815*. A genome scan was performed by Beh et al. (2002) using lines of sheep diverging for parasite resistance and detected a suggestive QTL for resistance to *Trichostrongylus colubriformis* in 20-week-old sheep on chromosome 6 and a point-wise significant peak for 27-week old animals. This group also identified one region on each of chromosomes 1 and 12 reaching the point-wise significance in 27-week-old animals. Different regions were detected as likely to carry genes for resistance although no region was statistically significant after correcting for multiple tests. Davies et al. (2006) genotyped naturally infected lambs to scan regions previously identified as candidates for either genes for resistance or genes for other economical traits to determine whether these candidate regions could be confirmed in an independent dataset. Evidence of linkage was found on chromosomes 2, 13, 14 and 20. A genome scan performed by Crawford et al. (2006) using divergent lines and naturally infected animals detected a significant QTL on chromosome 8. Ingham et al. (2008) studied the cytokines gene expression level by qRT-PCR in susceptible and resistant locks (against *H. contortus* and *T. colubriformis*) and observed that the innate period would be critical to the development of *h. contortus* resistance, whereas the acquired period would be critical to the development of *T. colubriformis* resistance. Moreover, TLR (Toll Like Receptor) are up-regulated in resistant animals in general. In Kenya, a genome-wide scan for QTL affecting GIN resistance (predominantly *H. contortus*) was undertaken using a double backcross resource family derived from Red Maasai and Dorper sheep. Highly significant QTL was identified on four chromosomes (3, 6, 14 and 22) for FEC and PCV. The most significant QTLs for parasite resistance and immune response were co-located at the same position on chromosome 22, suggesting a single causative mutation may be associated with these traits.

In cattle four QTL were found to be associated With FECs on BTA 7, BTA 9, BTA 14 and BTA 19. Studies on QTL affecting parasite resistance in cattle have been carried in USA (Gasbarre et al., 2002). The Angus cattle population was used for divergent selection programme. For genetic analysis of the DNA polymorphism more than 200 microsatellite markers were tested spaced at regular intervals (about 20 cM) across the entire genome. Preliminary results of QTL analysis show that an expected heterozygosity index was 50%, and 45% for polymorphism information content (PIC). These data suggest that parasite-resistance is related to acquired immunity, associated with the IFN-γ gene. The gene was also found as QTL for resistance to gastrointestinal nematodes in sheep. Coppieters et al. (2009) reported ITAGE gene within BTA 19 QTL influencing GI nematode burden in Dutch Holstein-

Friesian cattle under natural infection and is up-regulated in resistant cattle (Araujo et al., 2009). The fine mapping using SNP indicated that size of QTL region was around 3.3 cM on BTA 19. Li et al. (2007) reported that MIP-1α gene is over-dispersed in resistant cattle. The QTL discovered at BTA 9 corresponds to the arthologous regions in sheep lying on ORA 8 (Crawford et al., 2006), ORA 11 (Beh et al., 2002) found to have moderate significance. Important genes related to nematode resistance are likely to lie in these regions. GRO gene, coding for MIP-2 α is within the sheep ORA 6 QTL (Beh et al., 2002).

Results from QTL studies do indeed suggest polygenic control, implying that single genetic solutions are unlikely. Crawford et al. (2006) noted that "Our failure to discover more QTL suggests that most of the genes controlling this trait are of relatively small effect". Therefore, although there are many opportunities to breed animals for improved parasite resistance, there is much work to do at various levels. This includes characterization o genetic resources, definition of sustainable breeding objectives and definition and development of appropriate technologies like targeted single nucleotide polymorphism (SNP) assay. Although use of marker-assisted selection (MAS) would seem to be particularly beneficial for improving disease resistance in the developing world there is as yet no example of this technique being used in developing countries. In large part, the failure to use QTL information reflects the lack of investment in QTL mapping in the developing world. Such investment is needed not only to detect QTL that could be useful in genetic improvement programmes, but also to design improvement programmes, utilizing QTL information, that would be sustainable under developing world conditions. The necessary investment is not easily obtained, because no coherent case has been made for a cost-effective and sustainable strategy for using molecular genetic information for genetic improvement in the developing world.

Interferon gama gene (INF-γ): The typing of Soay sheep at a diallelic microsatellite locus located in the INF-γ which is associated with resistance to nematode infection in domestic sheep revealed that one allele conferred reduced FEC and increased levels of immunoglobin A in young Soay sheep (Coltman, 2001). Researchers at AgResearch, New Zealand and CSIRO, Australia reported that q arm of chromosome 3 showing evidences of segregation of QTL that influencing parasite resistance in sheep. The most likely known candidate gene in this region is IFN-γ, which has been mapped to 3q23 by in situ hybridization technique (Goldammer et al., 1996). The IFN-γ is a cytokine that plays an important role in the regulation of the immune response to parasitic infection (Wakelin, 1996) and is highly functional and positional candidate gene. Crawford and McEwen (1998) performed the detail scanning of q arm of chromosome 3 using four microsatellite makers located within IFN-γgene, including a diallelic microsatellite located in the first intron described by Schmidt et al. (1996). The study showed the significant difference between the frequencies of the resistant and susceptible alleles at the IFN-γ microsatellite locus. In addition to the microsatellite markers, Crawford and McEven (1998) identified 36 nucleotide substitutions and insertion/ deletions of 1 and 8 nucleotides in the DNA sequences of the complete gene (4842 bp) from resistant and susceptible individual. Coltman et al. (2001) reported the gene conferring resistance to gastrointestinal nematode parasite may be located at or near the ovine IFN-γ gene. The reduction in FEC was significantly associated with an allele at the microsatellite locus in the first intron of the IFN-γ gene in Soay sheep. The ovine IFN-γ_{126} allele was associated with increased parasite specific antibody (IgA) in lambs but not in yearlings.

Inclusion of disease resistance in breeding programmes

The logistics of including resistance to GINs in breeding programmes have been discussed by a number of workers (Albers et al., 1987; Bisset et al., 1994, 1996; McEwan et al., 1995; Morris et al., 1995; Piper and Barger, 1988; Pocock et al., 1995; Woolaston, 1994; Woolaston and Eady, 1995; Woolaston et al., 1996). The most appropriate way to combine information on the different traits in a selective breeding scheme is to construct a selection index (Cameron, 1997). The necessary information includes details of the traits one wishes to improve (the selection objective) and the economic value of these traits. The economic value is the additional financial benefit which will be

derived from an improvement in the trait. The major difficulty in constructing a selection index which includes nematode resistance is placing an economic value on the trait. As mentioned above, the advantages of nematode resistance are undoubtedly important but are difficult to estimate with precision. There are following particular issues that require special consideration in breeding for disease resistance / resilience:

- Genetic diversity within the breed.
- Purity of the indigenous breeds.
- Measures to safeguard and upgrade the genetic purity of resistant breed.
- Whether breeding is for resilience or for resistance.
- Severity of pathogen challenge.
- Concurrent infections, nutritional and other stresses.

It should be kept in mind that

- Inclusion of resistance in a breeding programme need not greatly compromise gains in other traits. The most resistant animals can also be found amongst those with the highest production index.
- Irrespective of relative economic values assigned to GIN resistance, optimum progress in parasite resistance will be between approximately 50% and 70% of maximum gain when considering other economically important traits.
- To reduce costs associated with measurement of disease resistance, multi-stage selection can be used. In case of resistance to internal parasites, it is preferable to cull for production traits at the first stage and resistance at the second stage rather than vice versa.
- Repeated measurement of resistance offers considerable advantages over a single measurement provided that it can be done cost-effectively, e.g., on a reduced number of samples.

SWOT analysis of genetic strategies for disease resistance

It has been observed that most of the benefits of incorporating genetic elements into disease resistance highlight the sustainability of worm management and the inherent low cost of genetic elements, once they are established.

Strengths:

- Genetic change is permanent
- Consistency of effect
- No cash input when established
- Prolong/protect the effectiveness of other measures
- Broad spectrum effects, i.e. increased resistance to one disease can increase resistance to others
- Low evolutionary change in helminths
- Adds to diversity of management strategies

Weakness:

- Goals of production system may change more quickly than genetic change can be implemented
- Uncertainty of genetic outcomes in different environments and production systems

- Need for some level of controlled breeding
- Cost of measurements and analysis
- Adds to technologies to be understood and implemented

Opportunities:

- Marketing of disease resistant stock
- Infrastructure which can be used for the other purposes, e.g. performance recording
- Mobilise communities for related activities including training and acquiring skills for farm and community management and marketing

Threats:

- Inappropriate stock may become cheap and/or widely available
- Genetic material may not be owned by local stakeholders
- Opposition/competition from existing investors in other control options, e.g. pharmaceuticals
- Problem in dissemination and adoption by farming communities

Potential economic impacts of inclusion of disease resistance strategy in worm management

The consequences of genetic change in the resistance of a population of animals to an infectious disease depend upon the transmission pathways of infection (Bishop and Mackenzie, 2003; Bishop and Stear, 2003). In GIN infections, there is a continuous flow of infection between the host population and the reservoir (pasture). The outcomes of selection should be measured at the population level, rather than the individual animal level. Moreover, the outcomes are very non-linear and depend upon the starting point. Genetic improvement which results in a reduction in the clinical signs of disease that is, improved tolerance of infection will be effective in reducing the incidence or the impact of disease in the target population. However, it may not decrease the prevalence of the pathogen. Hence, the disease incidence in other populations in the same environment will not be affected. The major features of the potential to exploit this genetic variation in disease resistance parasites are demonstrated in single-trait selection flocks. Such long-term flocks have been established for *H. contortus* (Woolaston and Piper, 1996), *T. colubrijormis* (Windon, 1991) and *Ostertagia* spp. (Cummins et al., 1991). In all cases of selection for resistance to GIN, a change in the FEC has been achieved in the desired direction (Woolaston and Eady, 1995), closely associated with reduced parasite burdens as a consequence of reduced establishment and parasite fecundity. The advantages of breeding for disease resistance are as follows:

- There will be fewer diseased animals in the flock which suffer adverse effects of parasitism
- Resistant animals will transmit fewer pathogenic agents, which means reduced pasture contamination
- The trait indicating resistance (FEC) is simple to measure and testing protocols can be designed to minimise both the period of infection and adverse effects on the host.
- Importance of disease resistance is more realistically seen in the long-term, when resistant flocks would require no or little disease management. Clearly, any increase in labour costs or a desire to decrease reliance on chemicals for treatment would favour the development of such easy care features.
- Annual cumulative benefits of £4.1m in the UK (a high-cost economy) once selection is as widespread as selection for performance traits.
- Annual benefits of $1.8m in Australia, $0.2m in Indonesia, $1.2m in India and $0.06m in Nepal, similar to the benefits from implemented non-genetic endoparasite control.

- Large returns on current research investment. It was estimated that the net benefits of current research in developing countries, over a 30 year time horizon were $387m, IRR = 78.9%, benefits : costs = 180:1 for a phenotypic measurement project and $160m, IRR = 40.7%, benefits : costs = 70:1 for a genetic marker project.

Future areas for research

The increasing parasitic infection coupled with problem of emergence of anthelmintic resistant strains of parasites in sheep is becoming serious problem in India or worldwide. In view of the above fact, there is an urgent need to develop sustainable strategies for control of parasites. Identification of genetically resistant animals could be facilitating, if the genomic loci responsible for the majority of the genetic variation in host resistance would be known. Identification of genes or markers which regulates the resistance will improve our understanding of mechanisms of host defense against parasites. There are few ways for improving the parasitic resistance; first, to identify the gene (s) or marker in the host, which is associated with parasitic resistance in sheep. Till date, there is no potential candidate gene has been identified that regulating the parasitic resistance with known mechanism except few genes (MHC, IFN-γ). If gene (s) of interest is identified than the DNA marker may help to select those animals and can be incorporated in to breeding programme. Secondly, production of transgenic animal may be another possibility, if a single gene (s) is responsible for the expression of parasite resistance, then transfer of such genes from resistant to susceptible individuals may provide a more long-lasting resistance. From the above discussions, first it is essential to identify the candidate gene conferring resistance to the parasites in sheep, then its transfer through transgenesis and finally expression into susceptible animals for enhancing the resistance level to the particular parasite. Establishing a nucleus flock based on proven genetic resistance that can act as a reference flock should be a priority. Pedigree, production and disease data in this reference flock should be recorded. Such information will make it possible to identify SNP in the breed.

Conclusions

The importance of maintaining and utilizing resistant / tolerant native breeds which until recently were neglected because these were often considered to be low productive, is receiving increasing attention. The genome research is a multidisciplinary approach and heavily influenced by new and improved technological advancement. The DNA microarrays and proteomics can provide a variable number of candidate genes for QTL. It is suggested that the multiple approaches are to be needed to identify positional candidate genes for parasitic resistance. Functional and comparative genomics and sequence analysis will also play an increasingly important role in facilitating the transfer of new knowledge from the best known model to small ruminants of economic importance. In summary, there are at least two *loci* that have shown associations with resistance of ruminants to gastrointestinal nematodes. One is the *DRB* gene, belonging to class II of the major histocompatibility complex. The other is a chromosomal region encompassing the interferon gamma gene. Both IFN-γ and *DRB* genes regulate immune responses to infections. Additionally, a number of QTLs for resistance to gastrointestinal nematode parasites in ruminants was identified. A community-based breeding approach could be one way to introduce selection for disease resistance in developing countries. However, in many developing countries, the prospects or the sustainable use and the development of local breeds are probably quite bleak. The main problems are linked to the absence of breeder's organization and institutional framework. There must be incentives for local breeds development and marketing and must support for the establishment of sustainable breeding programme and the creation of breeder's association. The impetus for pursuing the genetic approach for disease control is to eventually provide a means\to identify resistant animals through molecular markers. Unfortunately that goal is still away off as resistance is most certainly controlled by a number of genes which makes finding the right combination difficult. Thus, traditional breeding to select or resistant

animals or identifying and maintaining resistant breeds are the only means currently available. The key lesson from nematode resistance is that the total benefits from selection can be larger than those arising directly from genetic change in the host, i.e. there may well be additional environmental or epidemiological benefits as well.

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Application of Nanotechnology in Wool Processing

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Nanotechnology is an emerging field in all areas of science, engineering and technology and interdisciplinary in nature. Its practice requires physicists, chemists, biologists, material scientists and engineers to work together in close-knit teams. A nanometer is one billionth of a meter. The “nano” comes from a Greek word meaning dwarf. Nano science is concerned with making, manipulating and imaging materials having at least one dimension in the size range of 1-100nm and nanotechnology can be defined as a device or machine or product or process based upon individual or multiple integrated nano scale components (Geoffrey et al., 2009). The ideas and concepts behind nano science and nanotechnology started with a talk entitled “There's Plenty of Room at the Bottom” by physicist Richard Feynman at an American Physical Society meeting at the California Institute of Technology on December 29, 1959, long before the term nanotechnology was used. In his talk, Feynman described a process in which scientists would be able to manipulate and control individual atoms and molecules. Over a decade later, Professor Norio Taniguchi coined the term nanotechnology, in his explorations of ultra precision machining. The nanotechnology began momentum after the development of the scanning tunneling microscope during 1981 by which individual atoms could be studied (NNI, 2008).

Many properties of the solids depend on the size range over which they are measured. When a material is converted to nano size, the physical, chemical, mechanical electrical and magnetic properties are changed compared to their original material. The surface area of the nano material is very high and which imparts high reactivity and catalytic activity. For examples, the surface to volume ratio of 1 nm dimension cubic particles is one billion times the surface to volume ratio of 1 m dimension cube. These size dependant properties of the nano materials are used for several applications (Charles and Owen, 2003). In the case of textile fibres, the surface of characteristics of nano fibres with respect to conventional fibres is given in Table 1. Nano fibres have more than 10000 times surface area compared to the typical textile fibres.

Table 1. Characteristics of nanofibres (Satiskumar, 2011)

	Diameter	Typical specific surface area (m^2/g)	Surface area gain factor
Human hair	100 µ	0.041	1
Typical textile fibers	12-15 µ	0.410	10
Micro denier	1-2 µ	4.0	100
Nano fibers	10 200 nm	400	>10,000

Nano materials production

Two different approaches (Top-down and Bottom-up) are generally followed for the production of nano materials. In the top-down approach, large size particles are broken down to nano-sized particles by exerting very high energy. Ultrasonication, homogenization, ball milling, and laser ablation are some of the processes fall under this category. Though these processes are being commercially used, the size distribution of the resultant particles is very wide. In the bottom-up approach, molecules, ions are used as precursors and they are assembled to form nano-size particles by chemical and physical processes. The advantages of this approach are low energy requirement and

narrow size particle distribution which is considered as an essential in the application field. Chemical vapour deposition, chemical synthesis, sol-gel process, etc, are some of the examples of this approach.

The nano fibres can be produced by electro spinning process. Electro spinning is a cheap and relatively simple technique to produce nano fibres. This technique, though very old, has regained interest since it enables the production of cheap nanostructures. This process mainly consists of a DC power supply used to generate high voltage in the order of 25 to 85 kV between a polymer solution to be drawn out into nano fibres and a grounded collector plate (Fig. 1). The polymer solution to be drawn is stored in a syringe which has a thin capillary tube mounted at one end. The syringe is typically driven by a syringe pump at a specific flow rate. The spinning process is carried out by pumping the polymer solution to the end of the capillary tube such that it forms a small pendant or hemispherical surface. Then, a high voltage is applied between the polymer solution and the collector plate placed at a distance from the capillary tube. The electrostatic forces generated as a result of this electric field act against the surface tension and viscosity of the solution, thereby transforming the pendent like shape or the solution into a conical shape called the Taylor cone. Under further influence of the electrostatic forces, the polymer solution forming the Taylor cone stretches into a fine jet of fibre that travel towards the grounded collector plate and finally gets deposited on it. Continuous deposition of such fibres on the collector plate results in the formation of a nonwoven mat having high surface area-to-volume ratio, carbon nano tubes (CNTs) can also be prepared using this process (Karthick and Nadar, 2005)

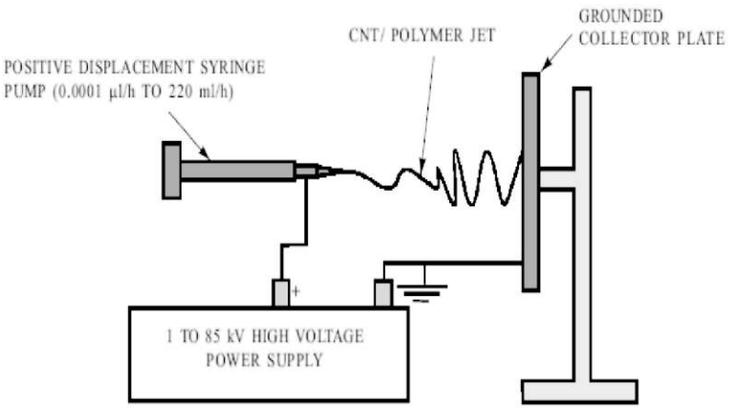

Fig. 1. Nano fibres production by electro spinning

Classifications of nano materials

Nano particles can be classified into quantum dots which is zero dimension, nano wires in which one dimension in nanometer range and quantum well (thin film) where the thickness of the film in nanometer range and three dimensional spherical nanoparticles. The commonly used nanoparticles are (Vigneshwaran, 2011):

1. Metal - Silver, Gold, Silicon
2. Metal oxide - Zinc oxide, Titanium dioxide, Iron oxide
3. Ceramic Ceria, Alumina
4. Polymers/organic Starch, Chitosan, Cellulose, PVA
5. Carbon Graphite, Single wall nano tube, Multi wall nanotube, Graphene

The other important form of nano particle is nano composites. The nano composite is defined as a composite material in which the matrix and/or reinforcing material is in nano size form. The nano composites exhibit increased

mechanical strength and stiffness of polymers while maintaining elongation compared to the conventional composites with significant weight reduction. Polymer nano composites are used in wide application like light weight and high strength materials, conductive polymers etc.

Characterization of nanomaterials

Though nanotechnology is the new field, nano particles were used for centuries. The nano particles are not visible to human eye. The nanotechnology research was possible after the invention of Atomic force Microscopy by Eric Drexler in 1986. The most important tools used for characterization of nano materials are atomic force microscopy, transmission electron microscopy, scanning electron microscopy, X-ray diffractometer and particle/zeta potential analyzer. Conventional UV-visible spectra, FT-IR etc can also used to qualitatively find out the nano particles. Atomic force microscopy is the ideal for quantitatively measuring the nano size particle in three dimensional structures with very high resolution. The transmission electron microscopy (TEM) works in the principle that when beam of electron is passes through the sample, the transmitted electrons form a two dimensional image. The magnification of the TEM is above 1,50,000. The scanning electron microscope (SEM) also uses electron beams compared to the light in the case of conventional microscopy. The SEM also produces two dimensional images with the magnifications of about 30,000. X-ray diffractometer can also be used to study the nano size materials with the help of Scherrer's formula. Particle size analyzer is used to study the size and distribution of nano materials using dynamic light scattering principle.

Nanotechnology application in wool processing

The application of nanotechnology in the wool processing can be classified into the following areas:

- Prickliness reduction and softness improvement
- Flame retardant
- Water and stain repellent and self cleaning
- Anti moth
- Antimicrobial
- UV protection
- Nano wool for different medical and industrial application

Prickliness reduction and softness improvement: One of the major problems pointed by the wearers against wool fabrics is prickliness. Prickliness is an unpleasant itching sensation when the wool fabrics are worn next to skin. This is caused by the coarse fibres above 32μ and also the presence of damaged broken fibre ends in the wool materials. Different methods like treatment with *Aloe vera* extract, microcapsules containing moisturizer, chemical agents, which reduces the fibre micron to import softness have been reported to solve the prickliness problem and to impart soft handle. Similarly, wool fibres were treated with proteolytic and lipolytic enzymes alone or in combination with oxidative chemicals for imparting softness in addition to shrink resistance character. With the advent of nanotechnology, several research studies have been conducted to resolve the problems. The majority of the research focused on application of nano-silica particles and nano silicone softening finishes. The wool fabric was made into more hydrophilic by the application of silica nanoparticle of 27 nm diameter. By this process, the handle of the fabric was improved with high softness. Silicone softeners have been applied to wool materials since long time to improve the softness of the wool materials. Silicones softners are chemically polydimethyl siloxane. Nano silicone softeners are able to penetrate into the inner side of the fabrics and provide durable softness to the fabrics.

Flame retardant finishing: Though wool is inherently flame retardant, it also required to be finished with flame retardant chemicals for certain high end applications like airline carpet, home textiles, fire fighter's uniforms and children wear. Conventionally, chemical additives (halogenated hydrocarbons) are being used to reduce the flammability of fabrics but these have a number of side effects. If the fire occurs, the halogen will produce highly toxic and corrosive combustion gases and also may create several environmental problems (Thilagavathi et al., 2008). Nanometer size clay particles (montmorillonite) based nanocomposite coating on wool makes it flame retardant. Nano-antimony, nano-borax etc will also be the better alternative for flame retardant finishing of wool.

Water and stain repellent and self cleaning: Medium and coarse wool fibres are mainly used for producing home textile materials like blanket, carpet etc. These types of fabrics need water repellent and stain repellent properties. At present water repellent finish is given by Teflon coatings. Nanoparticle based finishes in this category is promising. The silica and calcium carbonate nanoparticle composite with polystyrene (non fluorine compound) have been used to produce ultra hydrophobicity (Karthik et al., 2008). Another major development in this area is self cleaning fabrics using titanium dioxide nanoparticles. Self-cleaning fabrics containing titanium dioxide nanoparticles have been developed based on their photo catalytic ability of oxidizing dirt and other contaminants. These types of fabric can be put to both military and civilian uses. It is reported that the textile substrates like cotton, wool, polyamide and polyester were pre-treated with RF-plasma, MW-plasma or vacuum-UV irradiation in such a way that negatively charged titanium dioxide chelating groups, such as carboxylic groups, are introduced by the pre-treatment methods. Such titanium dioxide treated materials showed self-cleaning activity under daylight environment (Bozzi et al., 2005; Yuranova et al., 2006).

Antimicrobial and antimoth finishing: The most common application of nanotechnology to wool is antimicrobial treatments with silver nanoparticles. There is a growing interest among the consumer for fresh and hygienic textiles. Wool materials coated with silver nanoparticles are considered to kill odour causing bacterial and make them fresh. It is reported that wool fabrics coated with silver nanoparticles provide antimicrobial efficacy to both gram positive and gram negative bacteria and the finish is durable upto 20 launderings (Raja et al., 2010). These coatings in addition make the fabric self-wound healing, water-repellent, and dirt-repellent with the addition of polymers. Research is also going on to prepare the chemical and biological protective clothing with self-decontamination and drug-delivery characters. The decontaminants like nanometal oxides (MgO, Al_2O_3) along with activated carbon and antibiotics in the form of nanocoatings is being explored for this purpose (Hussain and Ramkumar, 2006).

Wool materials are prone to attack of moths like carpet beetle due to their keratin content. The protection of wool material is being done by using halogenated compounds. The development of antimoth finishing using inorganic nano particles is in research stage only. It was reported that a combination of sulphur-silver nanoparticles application to wool make it moth proof (Ki et al., 2007).

UV protection: UV protection finish to wool is necessary to prevent the harmful UV radiation which is carcinogenic in nature. Zinc oxide nanoparticles embedded in polymer matrices like soluble starch are a good example of functional nanostructures with potential for applications such as UV-protection ability in textiles. For this purpose, wool materials are treated with nano sized zinc oxide nanoparticles. Zinc oxide nanoparticles absorb the harmful UV-A and UV-B rays. It was reported that zinc oxide nanoparticles also provide antimicrobial finishing to textile materials in addition to UV protection (Vigneshwaran, 2011).

Nano toxicology

In spite of the excellent properties of nanoparticles, some scientists also expressed their concern with toxicology problems with nanotechnology products. They argued that these technologies have been recklessly released upon consumers without adequate testing and understanding of potential consequences. The unpredictable

and unknown side-effects of nanoparticles concern some scientists, environmentalists, and health advocates. The nanoparticles used in cosmetics or clothing may create toxins that are easily absorbed into the skin and circulatory system and, because of their very small size, be carried throughout the entire body including the brain, with unknown consequences. Further there may be possibility of nanoparticles for escaping and leaking into the environment during manufacturing processes also increases with unknown results.

Nano wool and keratin extraction

It was attempted to produce nano wool by grinding method (Wang et al., 2009) using the short wool and spinning waste. The nano wool has been used for different applications like coating on cotton and other synthetic fabrics, packaging film etc (Denning, 2009). The nano wool could be used for preparing composite materials for absorption of heavy metals, toxic gases, oil spills in sea etc. Wool in nano form is a very good absorber of heavy metals (like chromium, cobalt etc), toxic gases (like formaldehyde, methane etc) and oils. Attempts have been continuing to produce nano wool fibre through electro spinning process instead of grinding principle. The electro spun nano wool fibre and fibre mats has potential application as a filtration media to absorb very fine minute particles of materialsand can be used ballistic protection applications, electrostatic filter, bio-medical applications, toxic material absorption etc.,

Wool is composed of keratin polymer. Keratin is abundantly available from the poor quality coarse wool not suitable for spinning. The keratins obtained from the wool have potential usage in the fields of wound healing, drug delivery, tissue engineering, toxic material absorption, cosmetics, medical devices etc**.** The keratins can be used as a scaffold for development of biomaterials due to their intrinsic biocompatibility, biodegradability, mechanical durability, and natural abundance (Rouse and Van dyke, 2010). The keratins have the ability to form three dimensional porous structure and also cell binding sites which make keratin an ideal material for tissue engineering and biomaterial preparation. The keratin powder obtained from the burning of human hair has been used for wound healing purpose since ancient time by Chinese people. Similarly keratins can be loaded with drugs and can be used for slow releasing principle.

Conclusion

Nanotechnology based wool processing provide unique opportunity to increase the value of the wool products. Some of the process like antimicrobial finishing, water repellent finishing have been already adopted by the industries. The nano-finished woolen and worsted materials are getting more values due to their aesthetic and functional nature. Lot of new developments in the wool based nano materials and processes are in the research stage. The electro spinning process for nano wool fibre production is optimized and the products are very good opportunity in the field of biomedical application, filtration and toxic material absorption.

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Rumen Biotechnology: Implication in Animal Nutrition

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There is a massive transformation of world's agriculture in the last few decades due to higher demand in food for teeming human population. In similar line, the livestock sector has also undergone a tremendous transformation, fuelled by high demand for livestock products (milk, meat), and it is further expected that the demand is likely to increase world-wide and the trend of present scenario in developing countries is still promising, a further two fold increase in the demand of livestock product is expected. The major driving force behind this soaring demand for livestock products is a combination of population growth, urbanization and growth in income and/or increasing purchasing power. The challenge is to enhance animal productivity without any adverse effects on environment. Our country had witnessed the success of Green Revolution in the early 70's to become self-sufficient in food grain production, while White Revolution in dairy sector had boosted milk production. But the present trend in the alarming increase of human population has posed a serious challenge to increase food and livestock products for feeding to teeming millions. Further, the world's population is expected to grow to 7.5 billion people in 2020 with most of this growth occurring in developing countries (IFPRI, 2001). As a result, demand for animal products is expected to grow tremendously (Bradford, 1999). In view of this fact, the methods of livestock production that is being followed presently must be re-looked and necessary changes that are required in the existing production system must be adopted to allow for efficiency and improvement in livestock productivity.

The major limitation to ruminant production in many of the tropical countries like India can be attributed to poor nutrition. Ruminants like cattle, buffalo, sheep, goat etc. are reared generally on pasture-based feeding system, wherein, the animal thrives on grazing resources and on fibrous crop residues for meeting their nutritional requirement. The productivity of these animals on such diets is restricted due to lower nutrient contents, which is characterized by low protein, high fibre and low ME content. Technologies like chemical treatment of fibrous feedstuffs, supplementation of tropical roughages with leguminous fodder trees and shrubs and low-cost nitrogenous sources (urea), and use of agricultural by-products are promising methods to alleviate nutrient deficiencies associated with these basal diets. Sometimes feeds contain secondary plant metabolites (e.g., tannins, saponins, phenolic glycosides), which alter nutrient utilization by affecting discrete populations of microorganisms in the rumen. Detoxification technologies for ameliorating anti-nutrient content in these feedstuffs are also available to make them more wholesome and nutritive.

Manipulation of rumen ecosystem

Ruminant animals are exceptionally inefficient in their retention of nitrogen under conditions where the diet contains protein which can be fermented rapidly in the rumen. Protein is converted via peptides and amino acids to NH_3, which can be incorporated by ruminal microorganisms, but which, when present in excess, is absorbed rapidly across the ruminal wall. Proteolysis is carried out by many ruminal species (Morrison and Mackie, 1996; Wallace et al., 1997), and large variation between individual animals occurs (Depardon et al., 1996). Peptide catabolism is for the most part a two-step process, whereby oligopeptides are first cleaved to dipeptides and then broken down by dipeptidases to amino acids (Depardon et al., 1996; Wallace, 1996). The released amino acids are then deaminated by ciliate protozoa and two categories of ruminal bacteria: the first category is comprised of many of the most numerous species of ruminal bacteria, which possess a low deaminative activity, and the second category is comprised of a much smaller population of so-called

hyper-ammonia-producing (HAP) bacteria (1), which have much higher deaminative activity and which are believed to rely on amino acids rather than sugars for energy generation (Russell et al., 1988; Paster et al., 1993; Attwood et al., 1998). Inhibition of any of these catabolic steps would benefit nitrogen retention in ruminal fermentation. Wang et al. (2004) found that dipeptidyl peptidases inhibitors can influence the rate of NH_3 production in the rumen and may form the basis for developing protein-sparing feed additives for ruminants.

Feed additives and supplements: Present methods for manipulating ruminal fermentation that involve microbial biotechnology include dietary ionophores, antibiotics, and microbial feed additives (Wallace, 1994; Kamra, 2005). Adding specific nutrients to feeds improves animals' diets and lowers feed costs, e.g. amino acids can be produced in large quantities through fermentation, a biotech process for growing microorganisms. Similarly, enzymes are proteins and can be produced through large-scale fermentation processes, which aid specific chemical or metabolic reactions necessary for cell growth. They improve feed quality and allow feeds to be tailored to the specific needs of different animals. It also increases the animal's ability to digest the feed, e.g. cellulose, xylanase, -glucanase is added to the diets to utilize the non-starch polysaccharide from barley, oats etc and fibrous crop residues. Supplements are added to remove compounds naturally present in feed grains and forages which are potentially harmful to animals or which interfere with their nutrition, e.g. phytase is added to feed to improve digestion of phytate and reduce the need for phosphorus supplements and prevents its excess use in animal feed, thus minimizing the build-up of phosphorus in the environment. The use of hormones which act as switches and start body processes, e.g. growth hormone helps cows and pigs more efficiently convert feed nutrients to meat or milk and the biotechnological application of research involve use of specific amino acids to stimulate the natural release of growth hormone, to improve growth and meat production.

Antibiotics and growth promoters: The use of antibiotics as feed additives is controversial due to the potential development of resistant bacterial pathogens in food-producing animals which are exposed to the antibiotics and the resultant public health risk. Uses of growth promoting feed additives have been banned since 1999 with only four substances remaining in this group of feed additives. However, the growth promoters (especially chinoxalines) were most suitable for the prophylaxis of a microbial imbalance in the gastrointestinal tract. Therefore, the amount of prescribed antimicrobial drugs for metaphylaxis and therapy should be critically observed and one has to take into consideration the reasons for the use of antibiotics (growth promoters and therapeutics) or other "aids" (e.g. ZnO, Cu) in food producing animals (especially in beef-cattle, pigs and poultry) in "modern" production systems (Kamphues, 1999; Wegener, 2003). In this scenario, the matter for conflict is the contrast between a minimised use of antimicrobial substances, as science as well as general public demand, and the requirements of "modern" livestock industry (rationalisation, increase in performance, specialisation, concentration) and general economy (save of resources, lowering of production costs). There is also evidence that resistance of gut bacteria to antibiotics increases with increasing concentrations of penicillin in the milk fed to dairy calves (Langford et al., 2003). Collignon et al. (2005) argued that eliminating the routine use of feed antibiotics will improve human and animal health, by reducing the development and spread of antibiotic-resistant bacteria. However, as energy becomes more expensive there will be an expanded use of feed additives other than antibiotics that improve digestive characteristics of food animals and greater dependence on modeling digestion will be keys to maximizing genetics and diet.

Prebiotics, probiotics and synbiotics: The concept of probiotics and prebiotics supplementation in animal feeding has increased with the ban in the use of antimicrobial feed additives in animal feeding. Probiotics can be defined as microbial food/feed supplements that beneficially affect the host by improving its intestinal microbial balance. These additives are supplemented for enhancing the production, gut health and for reducing the incidence of neonatal diarrhoea in animals. In general lactobacilli are used for monogastric animals, whereas yeast cultures are used for ruminants and horses. Among the numerous purported health benefits attributed to probiotic bacteria, the (transient) modulation of the intestinal microflora of the host and the capacity to interact with the immune system directly or mediated by the autochthonous microflora, are basic mechanisms (de Vrese and Schrezenmeir, 2008).

Well-established probiotic effects are:

- Prevention and/or reduction of duration and complaints of rotavirus-induced or antibiotic-associated diarrhea as well as alleviation of complaints due to lactose intolerance,
- Reduction of the concentration of cancer-promoting enzymes and/or putrefactive (bacterial) metabolites in the gut,
- Prevention and alleviation of unspecific and irregular complaints of the gastrointestinal tracts in healthy people,
- Beneficial effects on microbial aberrancies, inflammation and other complaints in connection with inflammatory diseases of the gastrointestinal tract, Helicobacter pylori infection or bacterial overgrowth,
- Normalization of passing stool and stool consistency in subjects suffering from obstipation or an irritable colon,
- Prevention or alleviation of allergies and atopic diseases in newborns,
- Prevention of respiratory tract infections (common cold, influenza) and other infectious diseases as well as treatment of urogenital infections,
- Hypocholesterolemic effect, improvement of the mouth flora and caries prevention or prevention or therapy of ischemic heart diseases or amelioration of autoimmune diseases (e.g. arthritis),
- Insufficient or at most preliminary evidence exists with respect to cancer prevention.

Prebiotics on the other hand can be defined as non-digestive food/ feed ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacterial species already resident in the digestive tract and thus attempt to improve host health. Examples of prebiotics that are commonly used in livestock feeding include oligosaccharides such as fructose-, mannan-oligosaccharides or other oligomers. Other health effects of prebiotics (prevention of diarrhoea or obstipation, modulation of the metabolism of the intestinal flora, cancer prevention, positive effects on lipid metabolism, stimulation of mineral adsorption and immunomodulatory properties) are indirect, i.e. mediated by the intestinal microflora, and therefore less-well proven.

Synbiotics is the term used for a mixture of probiotics (live microbial feed additives that beneficially affects the host animal) and prebiotics (non-digestible food ingredients that beneficially affect the organism) and is now gaining importance due to possible additive effects on the above described phenomena.

Defaunation: Predation by small protozoa is by far the most important cause of bacterial protein turnover (about 90%) in the rumen, with autolysis, other lytic factors and endogenous proteolysis being of minor importance. The removal of protozoa from the rumen fluid had little effect on the breakdown of methanobrevibacter, while lysis of the non-methanogenic ruminal bacterium *Selenomonas ruminantium* decreased by over 70% (Newbold et al., 1996). Santra and Karim (2002) reported that even with similar DM, DCP and DE intake, N retention, body weight gain, average daily gain and feed conversion efficiency were better in defaunated than faunated lambs. Long-term defaunation increases the abundance of cellulolytic ruminococci and methanogens but does not affect the bacterial and methanogen diversity in the rumen of sheep (Mosoni et al., 2011). An important advantage of defaunation is the possibility of a single stain or species of ciliate refaunation with the aim of determine their participation ratio in the biochemical processes and their interaction with other species with the aim of feeding ruminants with the best diets to enhance productivity as a whole.

Rumen biotechnology

The exploitation of rumen functions by manipulation of the rumen microorganisms is not a new concept and researchers have been trying to develop various methods to chemically treat specific feedstuffs in order to modify their susceptibilities to digestion in the rumen, or to develop compounds that modify the rumen fermentation pattern by affecting the growth or metabolism of various rumen microorganisms. In spite of considerable efforts that are put in

these type of research, the levels of success have been rather minimal, which is partly due to the lack of knowledge or under estimation of the complexities in the physicochemical architecture of plant materials and of the rumen microbial ecosystem. Application of biotechnology principles in rumen manipulation can be a tool that has enormous potential to assist animal scientists to enhance rumen efficiency and animal productivity as a whole. With the rapid new developments in molecular biology, recombinant-DNA technology, and computer-based instrumentation over the last decade, there has been a renewed interest towards manipulation of the rumen fermentative processes via use of genetically engineered micro-organisms, particularly with species of rumen origin. Assuming that the large gaps in our knowledge on the genetic and biochemical aspects of these micro-organisms can be adequately fulfilled by research in the coming years, it is reasonable to expect that genetically engineered rumen micro-organisms will be available and will possibly be in commercial use in the years to come.

Rumen molecular techniques: Until recently our knowledge of rumen microbiology was primarily based on classical culture based techniques (isolation, enumeration and nutritional characterization) which probably only account for 10 to 20% of the rumen microbial population. Conventional culture-based methods of enumerating rumen bacteria are being rapidly replaced by the development of nucleic acid based techniques which can be used to characterize complex microbial communities. The foundation of these techniques is small sub unit (SSU) rDNA (16S rRNA) sequence analysis which has provided a phylogenetically based classification scheme for enumeration and identification of microbial community members. The 16S rRNA sequences in DNA extracted from a mixed digesta sample can be amplified by PCR using primers and the diversity and identity of the amplified 16S rDNA can be further analysed by several molecular techniques including: 1) restriction enzyme analysis of amplified polymorphic DNA (RFLP); 2) 16S rDNA based cloning, sequencing and probing; and 3) denaturing gradient gel electrophoresis (DGGE), temperature gradient gel electrophoresis (TGGE), and single strand conformation polymorphism (SSCP). Quantitative estimates of microbial populations can be performed by amplification of SSU rDNA with specific primers using real time PCR (RT-PCR). Also the more variable sequence regions of the 16S rDNA are hybridisation sites for genus, species and sometimes even strain specific hybridization probes.

In grazing ruminants that generally rely on highly fibrous diets as source of energy, not less than 50% of the dietary fibre passes through the digestive tract in an undegraded form (Cherney et al., 1991). In this situations *Butyrivibrio fibrisolvens*, *Ruminococcus albus*, *R. flavefaciens* and *Fibrobacter succinogeneses* are regarded as the primary fibre degrading bacteria in the rumen (Krause et al., 2003). The genetic diversity and phenotype of *Ruminococcus* have received little attention and very few investigations have been carried out in a systematic manner, but for other fibrolytic bacteria, a number of studies are available. DNA-DNA hybridization studies have shown that *Butyrivibrio* can be divided into at least five groups, suggestive of five different species. Comparatively a more recent 16S rDNA analysis has confirmed the DNA-DNA hybridization results of B. *fibrisolvens* and show that this bacterium is polyphyletic (Froster et al., 1996). Krause et al. (1999a) have undertaken a study to assess the genotype of 26 *Ruminococcus* strains isolated from cattle and sheep and their ability to digest plant cell walls. Only three of these strains had the same genotype and extent of fibre digestion of a medium quality grass varied from 0-61%. The genotype was based on ribosomal RNA sequence analysis. It is believed that if this phenotypic diversity is represented within an individual rumen, then increasing the number of highly fibrolytic *Ruminococci* could potentially improve the rates of cell wall digestion in the rumen. In another study, Krause et al. (1999b) collected the highly fibrolytic *Ruminococcus* strains and evaluated them for their ability to colonize the rumen and enhance fibre digestion. Tracking systems based on strain specific 16S rDNA sequences indicate that inoculated *Ruminococcus* strains did not persist for longer than 3 weeks before reaching undetectable levels. The resolution of this issue is fundamental to understanding the true contribution that individual strains make to fibre degradation and to the population of species and genera in the rumen and overall fibre digestion.

Methanogenesis provides a means of H_2 disposal and a mechanism to maintain low partial H_2 pressure in the rumen, a prerequisite for efficient ruminal fermentation. As methanogens possess stringent requirements for cultivation, only a few species have been successfully isolated from the rumen and cultured. Culture independent molecular based approaches have provided new insight into the diversity, prevalence and role of methanogens within the overall rumen microbial community. In addition, recent developments in next generation sequencing technology have enabled the ruminal microbial community to be investigated at a metagenomic level, providing information to explore the structure, function and metabolic diversity of microbial communities (Zhou et al., 2011).

Genetically engineered ruminal microorganisms: The effects of animals genetics on the rumen microorganisms is difficult to assess because of the confounding effect of diet on the composition in rumen. There are good prospects for manipulating rumen microflora to enable better utilization of feeds in ruminant species through the degradation of fibre and lignin, increasing the efficiency of nitrogen utilization and allowing the breakdown of anti-nutritional and toxic factors. The ability to adapt is essential if a particular microorganism is to remain prevalent in a variable rumen ecosystem. Factors governing the survival of new strains *in vivo* are ill-understood, and attempts to select in favor of added new organisms have so far been unsuccessful and therefore, in the short term, it may be advantageous to use nonruminal organisms, such as *Saccharomyces cereuisiae,* rather than indigenous ruminal species as a vehicle for implementing the benefits of recombinant DNA technology to ruminal fermentation (Wallace, 1994). According to Eggen (1994), although the molecular features of genes and expression signals in methanogens are well-studied, investigations on the regulation of gene expression in these organisms are very scarce. Gabriella and Eric (1997) advocated research on various fibrolytic enzymes and cellulose binding domains may allow for the transfer of novel genetic material to bacteria for enhancing the hydrolysis of plant cell walls.

The potential of recombinant DNA technology to develop new strains of bacteria for improved fiber digestibility remains largely unrealized and various strategies proposed have included the following: *1*) increasing the competitiveness of cellulolytic organisms (*F. succinogenes*, *Ruminococcus*) by conferring the ability to utilize xylose and pectins, thereby allowing earlier colonization of particulate matter; 2) inserting the cellulase gene into numerically predominant species (*B. ruminicola*); 3) increasing the competitiveness of cellulolytic species present in the rumen in low numbers (*C. polysaccharolyticum*) by according the ability to adhere to feed particles; 4) inserting an acid-tolerant cellulase gene into acid-tolerant bacteria (Lactobacillus) to allow fiber fermentation at a ruminal pH less than 6, 5) developing a cutinase activity in predominant bacteria; and 6) allowing predominant species to degrade arabinose side chains, thereby overcoming the rate-limiting effect of lignin. There have been at least 50 scientific reports on the cloning of genes coding for fiber-degrading enzymes (Wallace, 1994). The establishment of genetically modified microorganisms or 'foreign microbes' in the rumen can be monitored using competitive PCR methods and 16S rRNA-targeted oligonucleotide probes, which do not require culturing of microbes. These probes can also be used to allow characterization of rumen ecology, and such information can be used to develop more appropriate feeding strategies and also to allow a reduction in the emission of environmentally polluting gases, in particular methane. Broad host range plasmids and transposons have been used successfully to introduce new DNA into ruminal bacteria, as have shuttle vectors constructed as chimeras of plasmids from ruminal species and *Escherichia coli. Further,* the expression of the gene products(s) should be known to be nutritionally useful in vivo and the mechanisms have to be found for introducing and maintaining the new strain in the mixed ruminal population. McAllister (2000) observed an environmentally responsive adaptation of a predominant ruminal species, Fibrobacter succinogenes, to the presence of condensed tannins. This was manifested as the production of protective surface carbohydrate and the concentration of cellulolytic enzymes at the site of cell attachment. *W. succinogenes* was shown to be most effective to augment nitrate and nitrite reduction, and to reduce methanogenesis (Iwamoto et al., 2002). The HMG-CoA reductase inhibitors, mevastatin and lovastatin (ca. 10 nM), inhibited the growth of strains of rumen *Methanobrevibacter* (Wolin and Miller, 2006). They did not inhibit the growth of strains of *Ruminococcus albus*, *R. flavefaciens*, *Butyrivibrio*

fibrisolvens, *Fibrobacter succinogenes* and *Selenomonas ruminantium* which are essential for ruminal fermentation of cellulose, starch and other plant polysaccharides.

The use of biotechnology based techniques like inoculations of native and recombinant rumen microorganisms and microbial feed enzymes (Flint and Scott, 2000) has been tried. 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 that 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.

Development of genetically modified ruminal microorganism that would have superior fibre-degrading abilities: 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 endoglunases. 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 (Cocconcelli et al., 1992). Xue et al. (1997) were successful in introducing a xylanase gene from the anaerobic ruminal *fungus Neocallimastix patriciarum* into *Butyrivibrio fibrisolvens* and achieving secretion of the enzyme. Krause et al. (2001) constructed a recombinant *B. fibrisolvens* that expressed a xylanase enzyme from the ruminal fungus *Neocallimastix patriciarum*. The recombinant *B. 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 problem is the ability to introduce and maintain the new strain in the mixed rumen population.

Molecular regulation of ruminal epithelial proliferation: Gradual transition from diets with low to moderate fermentability to those with a high fermentability is required in order to minimize the risk for digestive disorders, such as ruminal acidosis (Bevans et al., 2005; Penner et al., 2007; Steele et al., 2009a), and associated disorders, such as liver abscesses (Nagaraja et al., 2005) and laminitis (Nocek, 1997). Because ruminants derive a significant proportion of their metabolizable energy supply via absorption of SCFA across the ruminal epithelia, it is important to understand mechanisms regulating changes in epithelial function during dietary adaptation. The complete sequencing of the bovine genome has provided a new opportunity to investigate regulatory mechanisms involved in ruminal epithelial function and adaptation at the molecular level. A recent review has described the molecular basis for digestive processes across the gastrointestinal tract in ruminants (Connor et al., 2010).

Feeding highly fermentable diets to ruminants as a strategy to increase energy intake increases short-chain fatty acid (SCFA) production and reduced ruminal pH associated with highly fermentable diets which imposes a challenge to the metabolism and the regulation of intracellular pH homeostasis of ruminal epithelia. The ruminal epithelia respond to these challenges in a coordinated manner. Whereas the enlargement of absorptive surface area is well documented, emerging evidence at the mRNA and transporter and enzyme activity levels indicate that changes in epithelial cell function may be the initial response. It is not surprising that gene expression analysis has identified pathways involved in fatty acid metabolism, ion transport, and intracellular homeostasis to be the pathways dominantly affected during adaptation and after adaptation to a highly fermentable diet. These findings are important because the intraepithelial metabolism of SCFA, particularly butyrate, helps to maintain the concentration gradient between the cytosol and lumen, thereby facilitating absorption. Butyrate metabolism also controls the intracellular

availability of butyrate, which is widely regarded as a signaling molecule. Current data indicate that for butyrate metabolism, 3-hydroxy-3-methylglutaryl-CoA synthase and acetyl-CoA acetyltransferase are potential regulatory points with transient up- and downregulation during diet adaptation (Penner et al., 2011). In addition to nutrient transport and utilization, genes involved in the maintenance of cellular tight junction integrity and induction of inflammation have been identified as differentially expressed genes during adaptation to highly fermentable diets. This may have important implications on ruminal epithelial barrier function and the inflammatory response often associated with subacute ruminal acidosis. The molecular regulation of ruminal epithelial proliferation seems to be mediated through growth factors, such as epidermal growth factor (EGF) and insulin growth factor (IGF-1) (Baldwin, 1999). Future studies should evaluate the linkage between these growth factors and their receptors to elucidate the mechanisms involved in epithelial proliferation. The increased ruminal ketogenic activity for cows fed highlyfermentable diets corresponds to increased mRNA abundance for Acetyl-CoA acetyl transferase and 3-hydroxy, 3-methylglutaryl CoA synthase, both of which are thought to be rate limiting enzymes for ketogenesis (Lane et al., 2002) and decreased expression of genes involved in cholesterol synthesis. Until recently, the cholesterol synthesis pathway has been overlooked in ruminal epithelia and, thus, future studies should evaluate the relationship between cholesterol synthesis, dietary adaptation, and inflammation. Moreover, the regulatory events controlling these changes have not been elucidated in ruminal tissue. In addition to changes in proliferation, and cellular activity, there is evidence that epithelial barrier function increases with increasing diet fermentability. Past studies have revealed the localization of claudin-1 and zona occludin-1 in the stratum granulosum but, the role of these tight junction proteins in the adaptive response remains to be elucidated. Further information regarding formation of tight junctions could be used to develop strategies to minimize the potential for the translocation of bacteria and toxins across the ruminal epithelia.

GM crops to harvest desired nutrients: Gene-based technologies have the potential to improve the nutritive value of ruminant feedstuffs that are fibrous, low in nitrogen and contain anti-nutritive factors. It is 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. Feeds derived from GM 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 enhance N utilization efficiency through decrease N excretion. Increasing the levels of amino acids in grain means that the essential amino acid requirements of can be met by diets that are lower in protein.

Nutrient drainage and environment

The fermentation of dairy effluent with yeast and bacteria and that of waste effluent with algal fermentation result in single cell protein production, which can be used as supplementary food/feed (Singh, 2004). Improvement of microbial strains by selection or genetic alteration and the potential of the agricultural byproducts and other wastes should be exploited. The contribution of animal nutrition to global warming is inefficient utilization of protein and energy in the rumen and resultant excess excretion of carbon dioxide, methane and ammonia to the environment. Biotechnological interventions can play a vital role not only in the productions but also in environmental protection and sustainability. Genetically modified feeds can improve water and soil quality by reducing levels of phosphorus and nitrogen in animal waste and lower green house gases by reducing ruminal methane production. Biotechnology, feeding, management and breeding can be combined to improve animal production and it may be possible to reduce methane generation by up to 60% (Singh et al., 2008). Biotechnology will contribute through the development of plants with tissues that are more digestible, animals that have improved digestive capabilities, and manure with a composition that better meets the nutrient requirements of plants. Some of the recommendations are as below:

- Combine the nutritional advantages of different transgenes into one plant to tailor animal diets for improved digestion and better nutrient management.
- Determine the limiting nutrients in cereal and plant cell wall digestion to provide robust basis for the genetic modification of forage plants and of ruminal microorganisms.
- Continue the development of hydrolytic enzyme that can be added as feed additives to improve digestion.
- Develop transgenic food animals with enhanced digestive processes, without negative impacts on either the welfare of the animal or the environment.
- Develop educational modules about the biology, biotechnology and social and ethical issues of manure nutrient management in animal agriculture.

Intervention for reducing methane emission

During the last decades, considerable research on methane production in the rumen and its inhibition has been carried out. Since methane production represents a significant loss of gross energy in the feed (2-15%), the ultimate goal of such intervention in rumen fermentation is i) an increase in feed efficiency; ii) its role in the global warming phenomenon and in the destruction of the ozone layer. The objective can be reached by intervention at the dietary level by ration manipulation (composition, feeding level) or by the use of additives (e.g. polyhalogenated compounds, ionophores and other antibiotics) or supplements (lipids, phyto-nutrients). More biotechnological interventions include reducing methanogenesis, defaunation, probiotics and introduction of reductive acetogenesis in the rumen (Weimer, 1998).

Methanogens belong to the kingdom of Euryarchaeota in the domain of Archaea and are characterized by their ability to produce methane under anaerobic conditions. Reduction or elimination of methanogenesis in the rumen has been touted as a way of improving animal production and may marginally benefit to control of anthropogenic release of methane.

However, drastic inhibition of methane production is not unequivocally successful as a result of several factors, such as: instantaneous inhibition often followed by restoration of methanogenesis due to adaptation of the microbes or degradation of the additive, toxicity for the host animal, negative effects on overall digestion and productive performance. Therefore, methanogenesis and its inhibition cannot be considered as a separate part of rumen fermentation and its consequences on the animal is also of paramount importance.

Reductive acetogenesis

Induction of reductive acetogenesis in the rumen or reutilization of produced methane through oxidation would result in a decreased release of gas in environment. In the hindgut of termites and mammals, acetogenic bacteria produce acetic acid causing hydrogen sink in hindgut fermentation:

$$2CO_2 + 8H \rightarrow CH3COOH + 2H_2O$$

Although hydrogen oxidizing acetogenic bacteria are present in the rumcn of sheep and cattle, they grow fermentatively on organic substrates. If metabolism of these indigenous acetogens could be changed from organotrophic to H_2 oxidizing, CO_2 reducing and consequently resulting acetate production, and if they can compete successfully with the methanogens for metabolic hydrogen, nlethane production would at least be reduced. Another possibility with the same result would be the introduction through the inoculation process (e.g., massive infusion of hindgut contents in the rumen of a cannulated animal). Competition of methanogens for hydrogen could be excluded by addition of specific methane inhibitors such as bromoethanesulfonate, sodium sulphatc, molybdate,

anthraquinone. Yeast powder etc has been reported to be effective. There is strong possibility that under such conditions the reductive acetogenesis is likely to increase. The dynamics of methanotrophs in rumen is poorly understood. Different behavior of relations between methanogens and acetogens in rumen and hindgut are probably due to differences in physicochemical characteristics of both ecological environment and fermentation substrate available. Only a better understanding of the effect of these conditions in the rumen and hindgut, determining the final result of the competition between methanogens, methanotrophs and acetogens in the rumen, can result in a successful introduction of reductive acetogenesis in rumen fermentation.

Redirection of reducing equivalents from methanogens to acetogens may be another way of decreasing ruminal methane production (McAllister and Newbold, 2008). Acetogens from a broad range of taxonomic groups have been isolated from various ruminants, including sheep, deer, bison and cattle (Joblin, 1999). Population densities of acetogens in ruminal fluid appear to be highly variable, ranging from non-detectable to 10^5/ml, but acetogens and methanogens co-exist within the digestive tract of humans (Robert et al., 2001), swine (De Graeve et al., 1994) and rodents (Dor´e et al., 1995). Considering co-existence of acetogens and methanogens in some mammals, it is unclear at this point why methanogens can so effectively out-compete acetogens for hydrogen in the rumen. Only two of six strains of acetogens assessed caused a modest reduction in methane emissions when added to mixed cultures of ruminal fluid (Lopez et al., 1999). Even if conditions in the rumen can be altered to favour acetogens, there is no guarantee that they will utilize hydrogen as obligate ruminal hydrogenotrophs have yet to be identified. Consequently, a multi-factorial approach to methane mitigation involving inhibition of methanogens, provision of alternative electron acceptors and development of low methane-emission diets may be required to bring about a meaningful reduction in methane emissions from ruminants.

Exploring positive nutritional attributes of plant anti-nutritional factors/plant secondary metabolites

The use of secondary plant metabolites (PSM), which were earlier considered as anti-nutritive, have enormous potential to modify rumen fermentation beneficially. Beneficial effects of PSM like tannins, saponins in ruminants include lower methanogenesis, anti-parasitic effect, increase milk yield, immune-modulation and so many other beneficial effects. The use of essential oils as additives in ruminant ration can also be one of the means to enhance rumen/gut metabolic efficiency, nutritional health and livestock products. Many essential oils, such as those present in cinnamon (cinnamaldehyde), clove (eugenol), garlic, oregano, and other plants, have antibacterial properties that have long been known and are used at high doses for medicinal purposes and food preservation. In the recent years, studies on several essential oils have revealed the potential of these molecules to increase milk production efficiency of dairy cows, and many producers have already started to implement essential oils into their feeding programs. Specifically, cinnamaldehyde and eugenol are currently being used by many dairy producers in developed countries. These feed additives have been established as safe for use, as they do not leave residues in milk or meat, and the beneficial effects are often comparable with other feed additive products on the market. Feeding essential oils to dairy goats and sheep has been reported to improve animal health by killing intestinal parasites and worms, increase milk fat and milk protein yield, improve udder health and decrease somatic cell count, reduce body fat mobilization thereby preventing ketosis. It also improves nitrogen utilization efficiency and reduces methanogenesis, thereby contributing to abating green house effect.

Essential oil supplementation

In the last few years, there has been an increasing interest in exploiting natural products as feed additives to solve problems in animal nutrition and livestock production (Wallace et al., 2002).The use of essential oils as additives in ruminants can be a mean to enhance livestock products and health conditions. Essential oils are compounds that give plants and spices their color and scent. Essential oils (EO) are steam-volatile or organic solvent extracts of plants

(Gershenzon and Croteau, 1991). The term 'essential' derives from 'essence', which means smell or taste, and relates to the property of these substances of providing specific flavors and odors to many plants (Calsamiglia et al., 2007). They are characterized as having a very diverse composition, nature, and activities. The most important active compounds present in essential oils are broadly included in two chemical groups namely terpenoids (monoterpenoids and sesquiterpenoids) and phenylpropanoids. They are mostly obtained from herbs and spices, and also present to some extent in many plants for their protective role against microbes (bacterial, protozoa or fungal) or insect attack. Structurally they are mainly cyclic hydrocarbons and their alcohol, aldehyde or ester derivatives.

Anti-nutrients and detoxification

Many conventional and un-conventional feedstuffs posses a number of antinutrients and toxic principles which retard their use in the livestock ration. The complexity of the rumen ecosystem makes it difficult to measure genetic adaptations in individual species of microorganisms. Undoubtedly, genetic adaptation by rumen microorganisms is what enables ruminants to acquire increased tolerance to toxins such as mycotoxin, mimosine and nitrate. Presumably, microorganisms shift their metabolism from substrates that are commonly available in the rumen towards toxic substrates which are occasionally available for metabolism. Jones and Megarrity (1986) successfully transferred dihyroxypyridine-degrading bacteria from Hawaiian (USA) goats to Australian ruminants to overcome the toxicity of *Leucaena,* a promising tropical browse leguminous plant possessing the toxic amino acid mimosine. Similarly, there is promise for tannin tolerant rumen bacteria which can be isolated from feral goats or other ruminants (Odenyo and Osuji, 1998; Wiryawan et al., 1999) exposed to prolonged feeding of tannin rich browses or tree forages and tried for establishment in animals which are exposed to similar high-tannin rich feedstuffs.

Manufacturing consumer preferred products

Another manipulation that can be made in the ruminant system is to increase the concentration of conjugated linoleic acids in livestock products like milk and meat. Food products from ruminants are the major dietary source of conjugated linoleic acids (CLA) for humans. The uniqueness of CLA in ruminant fat relates to the biohydrogenation of dietary unsaturated fatty acids by rumen bacteria. The CLA are intermediates in the biohydrogenation, and a portion escape the rumen and are incorporated into milk fat and body fat. In addition, the animal itself synthesizes cis-9, trans-11 CLA from trans-11 octadecenoic acid, another intermediate in ruminal biohydrogenation that is absorbed. This involves Δ9-desaturase, which is present in mammary tissue (lactation) and adipose tissue (growth).

Nutrigenomics and nutrigenetics

The recognition that nutrients have the ability to interact and modulate molecular mechanisms underlying an organism's physiological functions has prompted a revolution in the field of nutrition, i.e. the relationship between genes and diet. Research on molecular interactions of foodstuffs have indicated that gene expression is modified by a number of dietary components, including macro components (carbohydrates, proteins, fats and cholesterol), vitamins (Vitamin A, B, E, D) minerals (Fe, Se, Ca) as well as phytocompounds, including flavonoids, isothiocyanates and indoles (Kaput and Rodriguez, 2004). The unveiling of human genome sequence (Lander et al., 2001; Venter et al., 2001) in the 21st century opened a new era in nutrigenomics research. The creation of nutrigenomics and nutrigenetics, two fields with distinct approaches to elucidate the interaction between diet and genes but with a common ultimate goal to optimize health through the personalization of diet, provide powerful approaches to unravel the complex relationship between nutritional molecules, genetic polymorphisms, and the biological system as a whole. The integration of genomics into nutritional sciences has illuminated the complexity of genome responses to nutritional exposures while offering opportunities to increase the effectiveness of nutritional interventions, both clinical and population based. Nutrients elicit multiple physiological responses that affect genome stability, imprinting,

expression, and viability. These effects confer both health benefits and risks, some of which may not become apparent until later in life. The study of how genes and gene products interact with dietary chemicals to alter phenotype and, conversely, how genes and their products metabolize nutrients is called nutritional genomics or "nutrigenomics" (Kaput et al., 2005). It is expected that nutritional genomics will be a key area in nutritional science research over the next decade (Trayhurn, 2003) and that nutrigenomic studies will be very useful for elucidating the role(s) of food components in obesity (Chadwick, 2004), coronary heart diseases (Talmud, 2004) and cancer prevention (Davis and Hord, 2005) in humans. And similar application can be made in livestock research for enhancing productivity and prevention of diseases. Nutritional genomics challenges us to understand the reciprocal and complex interactions among the human genome and dietary components in normal physiology and pathophysiology. Understanding these interactions will refine current definitions of benefit and risk and lead to the establishment of dietary recommendations that have a high predictive value, minimize the risk of unintended consequences, and account for the modifying effects of genetic variation (Astley and Elliott, 2007). Furthermore, nutritional genomics will enable the design of effective dietary regimens for the prevention and management of complex chronic disease.

The nutritional needs of farm animals with respect to energy, protein, minerals and vitamins have long been known, and these have been refined in recent decades. Various requirement determination systems exist in different countries for ruminants and non-ruminants, which were originally designed to assess the nutritional and productive consequences of different feeds for the animal once intake was known. However, a considerable body of work exists associated with the dynamics of digestion, and feed intake and animal performance can now be predicted in many livestock species with high accuracy. A large agenda of work still remains concerning the robust prediction of animal growth, body composition, feed requirements, out put of waste products from the animal and production costs. Such work could go a long way to help improve the efficiency of livestock production and meeting the expectations of consumers and the demands of regulatory authorities. The development of nutrigenomic studies in the recent years has brought about a number of new research tools (transcriptomics, proteomics and metabolomics), which are important in animal nutrition and food research. Advances in genomics, transcriptomics, proteomics and metabolomics will continue to contribute to the field of animal nutrition and predictions relating to growth and development (Dumas et al., 2008). Better understanding of the processes involved in animal nutrition could also contribute to improved management of some of the trade-offs that operate at high levels of animal performance, such as those associated with lower reproductive performance (Butler, 2000).

Transcriptomics

Transcriptomics determines the level of all or a selected subset of genes based on the amount of RNA present in tissue samples. Transcriptomics is concerned with the expression of over 30000 genes in humans (Müller and Kersten, 2003). In precise experiments conducted on animals, the scope of investigation is usually restricted, for example the influence of dietary components on the transcript level is generally studied on selected organs. The use of a microarray containing probes for the over 8000 genes present in the liver of rats demonstrated that about 33% of the genes of rats fed a soya protein diet differed from those of casein-fed animals (Takamatsu et al., 2004). Significant differences were observed in the gene cluster concerned with lipid metabolism, and in the gene related to energy metabolism, transcription factor, and anti-oxidization enzymes. In similar experiments carried out by Tachibana et al. (2005), compared with casein, soya protein changed the expression of 120 genes involved in lipid metabolism, antioxidant activity and energy metabolism. Endo et al. (2002) also reported that various dietary protein sources resulted in a difference in expression of about 281 genes in rat liver, suggesting a nutritional function for protein components.

In the experiments of Collins and Hu (2007), microarray techniques were used to examine changes in gene expression in the rat duodenum associated with iron-deprivation. The findings demonstrated that iron-deprivation

results in a large spectrum of differentially expressed genes in the duodenal epithelium: the identification of these genetic changes is likely to increase our understanding of the complex physiology of intestinal iron homeostasis. The results of later experiments demonstrated similarities and provided evidence that more distal gut segments also may play a role in increasing iron absorption in iron-deficiency anaemia.

In the context of nutrition and micronutrient research in livestock species, transcriptomic methods have been popularly applied; however, it has been widely discussed albeit primarily in other studies using cell lines and animal models. Under such type of approach, a multitude of genes regulated at the mRNA level by dietary components has been identified and this, in turn, has provided new insights into the biological processes affected by nutritional parameters. In livestock species, the major application of nutrigenomics tools is to how effectively being utilized for dairy and meat industries. In dairy industry, an effective utilization of microarray technology was beneficial to study mammary gland tissues (milk production and udder health), muscle growth and development and myogenesis process (beef production) and the role of gut microflora on nutritional diet intake in ruminants (health and food safety). Study of Ron et al. (2007) has effectively been hybridized Affymetrix microarray (MG-U74v2) in identification of 249 differentially expressed probe sets common to the three experiments along the four developmental stages of puberty, pregnancy, lactation and involution. In context to candidate genes for milk production traits, a total of 82 expressed genes were identified in mammary gland tissue with at least 3-fold expression over the median representing all tissues tested in GeneAtlas.

Proteomics

Proteomics is the study of all the proteins in a particular cell, tissue or compartment (Banks et al., 2000). The major tools of proteomics are two dimensional (2D) gel electrophoresis and mass spectrometry (MS). Proteomics is concerned with over 100 000 proteins in humans (Müller and Kersten, 2003). In experiments on animals, the scope of the investigations is usually restricted to assessment of the influence of dietary components on the proteom of selected organs, for example, the liver. The proteome represents the protein equivalent of the genome, which is determined by the sequence, the type and number of its nucleotides. In contrast to this static nature of the genome, the proteome represents a tremendously dynamic object, which is influenced by a variety of parameters. However, arraying of proteins is more difficult than the arraying of DNA, because they have to maintain their correctly folded conformations. The fabrication of protein arrays is, therefore, particularly challenging and protein arrays have lagged behind so far in development because of the more complex coupling chemistry, the instability of the immobilized protein and the far weaker detection signals (Chipping, 1999). In contrast to these technical problems, genome-wide screens for protein function are of biological importance for many applications such as: analysing protein expression profiles, monitoring protein-protein interactions, identifying protein posttranslational modifications, screening the substrates of protein kinases, examining the protein targets of small molecules, and proteomic analysis as a function of bioprocess cultivation conditions.

The improvement of proteomics is crucial and several techniques are currently available to provide absolute and relative protein quantification (Elliott et al., 2009). Application of large scale proteomics in livestock has been useful for studying proteins composing milk fat globules and bacteria present in milk (Gagnaire et al., 2009), milk proteome after mastitis (Boehmer et al., 2010), and muscle quality (Picard et al., 2010). The study of proteomics in livestock will probably benefit by the development of protein-specific antibody arrays (Chen et al., 2003) or ImmunoCell-Arrays (Giorgetti et al. 2007). However, the advancement of these technologies is challenging because of the limited availability of specific antibodies in livestock species.

Metabolimics

Metabolomics represents the final step in understanding the function of genes and their proteins. The aim of metabolomics is to determine the sum of all metabolites (other substances than DNA, RNA or protein) in a biological system: organism, organ, tissue or cell (Müller and Kersten, 2003). Techniques employed to investigate the metabolome include nuclear magnetic resonance (NMR) spectroscopy, high performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS). These methods are capable of resolving and quantifying a wide range of compounds in a single sample. The main characteristics of these new technologies were miniaturization, automation, high throughput and computerization (Corthesy-Theulaz et al., 2005).

Metabolomics is concerned with several thousands of metabolites in humans (Müller and Kersten, 2003). As in the case of transcriptomics and proteomics, the scope of metabolomic analysis is mainly restricted to the assessment of the influence of dietary components on the metabolome of selected organs or tissue in animal nutrition studies. In experiments performed by Bertram et al. (2006) metabolomic analysis was implemented to detect the changes in the biochemical profiles of plasma and urine from pigs fed with high-fibre rye bread. Two diets with similar levels of dietary fibre and macronutrients, but with contrasting levels of wholegrain ingredients, were prepared from whole rye and fed to pigs. Using an explorative approach, the studies disclosed the biochemical effects of a wholegrain diet on plasma betaine content and excretion of betaine and creatinine.

In another experiment, proton nuclear magnetic resonance microscopy (H-1-NMR) was used to determine the metabolite profi les in the liver of rats used as a animal model to characterize the toxicity of triazol fungicides (Ekman et al., 2006). Triazole fungicides, which exhibit their antifungal activity by inhibiting fungal ergosterol biosynthesis, are economically important agricultural chemicals as they are widely used on crops such as wheat, barley and orchard fruits (Filipov and Lawrence, 2001). For this reason animal feed can be, sporadically, contaminated with these fungicides. The results of above quoted report support the possible application of a metabolomics approach to assess the toxicity of triazole fungicides and identifying biomarkers of exposure and/or effect.

New research/thrust areas of work

The Department of Biotechnology, Government of India has identified some of the broad areas where biotechnological tools can be exploited to improve the efficiency of utilization of feed and fodder viz. (i) genetic manipulation of rumen microbial ecosystem for improving productivity and protecting environment (ii) identification of genes for better feed conversion of agro-byproducts (lignin, oil cakes, oil meals, cellulose etc.) (iii) microbial feed additives (probiotics) for enrichment of feed quality (iv) use of fibrolytic enzymes in feed to improve digestibility and nutritive value and (v) genetically modified silage bacteria.

Application of biotechnology can be envisaged for improving the performance of animals through better nutrition, enhanced production potential or improved health status. 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. 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. Administration of recombinant somatotropin (ST) results in accelerated growth and leaner carcasses in meat animals and increased milk production in dairy cows (Singh et al.,

2008). Immunomodulation can also be used for enhancing the activity of endogenous anabolic hormones. A lot many areas in animal nutrition may encompass application of modern biotechnological tools which are detailed below.

Rumen manipulation: molecular and biotechnological approach

- Study on nutritional ecology in changing environment and diet diversity,
- Manipulation of rumen function and use of Biotechnological tools for improving rumen function & augmenting efficiency of nutrient utilization,
- Utilization of plant biomass containing bioactive components as rumen fermentation modulators for enhanced animal productivity: exploring pro-nutritional effect.
- Exploring rumen microbial species and identification of genes for better feed conversion of agro-byprod ucts
- Rumen manipulation and its related impact on meat composition, livestock production and human health

Nutrigenomics to enhance livestock production

- Manipulation of genome expression by nutritional intervention
- Exploring molecular dietary targets to understand diet-disease relationship, reproductive health, immune potentiating effects, antioxidant support etc.
- Specific biochemical markers to assess the micronutrient status of animals

Nutrition for designer/dietetic livestock products (meet and meat products) for better health

- Nutritional intervention to lower saturated fatty acids (SFAs) and raise monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) in meat and meat products
- Use of high forage based diets or oil/oil seed supplements to enhance n-3 PUFA in meat and meat products so as to support human health and reduce risk of diseases
- Dietetics for nutritional wellbeing and supporting health and amelioration of diseases

Nutrition, environment and nutritional toxicology

- Problotics/prebiotics in enhancing nutrient utilization and supporting health & wellbeing of animals
- Manipulation of nutrient-environment interaction for reducing green house gases emission from livestock
- Study on toxicological limits of plant antinutritive factors/plant secondary metabolites and exploitation of their potential for nutrient utilization, lowering methane and ammonia emission
- Plant-genetic approach for the development of new forage species and their evaluation for maintaining nutritional health (anti-b loat, anti-parasitic, anti-protozoal) and sustaining production in livestock
- Nutritional strategies to reduce the toxic effects of phyto- and mycotoxins

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Biotechnological Approaches for Enhancing Sheep Production

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Developing countries like India are faced with the challenge to rapidly increase agricultural productivity to ensure sufficient availability of food for their growing populations without depleting the natural resources. Biotechnology is regarded as a means to meet both the objectives through addressing the production constraints of small-scale or resource-poor farmers who contribute more than 70% of the population in India. In our country, the demand for meat has increased tremendously and emphasis has shifted from wool towards mutton as the main produce from sheep farming. There is acute shortage of meat for domestic purpose besides huge demands in the international market. Intensive efforts have been made to improve the productivity potential of the Indian sheep breeds mainly through the intervention of biotechnological tool and techniques.

Reproductive biotechnology

In India, there are about 71.56 million sheep with a vast biodiversity deriving from 42 described breeds. The productivity of native sheep breeds is a limiting factor to fill the gap between demand and availability thus necessitate urgent need to increase the meat production to fulfill nutritional demands of our huge population. The heavy demand can be met by applying integrated approaches for increasing reproductive efficiency in conjunction with biotechnological tools. The reproductive efficiency of Indian sheep breeds is relatively low, because they are raised under unfavorable and primitive management conditions[2]. Mostly, Indian sheep breeds have a long lambing interval and low fecundity. Reproductive biotechnologies including artificial insemination, multiple ovulation and embryo transfer, early pregnancy diagnosis and *in vitro* production of embryos may help in augmenting sheep production. In order to achieve desired improvement in genetic makeup of animals, incorporation of beneficial gene particularly of superior animal is required, which is very difficult through conventional breeding programmes. Recently developed cloning techniques such as nuclear transfer and transgenesis may have wider applications in sheep production in time to come. This article briefly describes the reproductive biotechnologies that have been developed and applied over the past decades in sheep improvement. Although, some of the reproduction techniques were developed in the country but their field level dissemination is very low.

Artificial insemination: Artificial insemination (AI) is being used widely for genetic improvement of sheep, goat, cattle, pig and poultry in the developed countries. It increases the male selection intensity and hence increases the average genetic merit of offsprings. Globally more than 3.3 million AI in sheep and 0.5 million in goat are performed annually, but only in very few developing countries where AI is practiced to a level that impacts substantially livestock production. Several methods have been developed for AI in sheep such as vaginal, cervical, trans-cervical and laparoscope aided intrauterine. Among these techniques, laparoscope aided intrauterine AI radically improves the fertilization rate with frozen thawed semen and has potential for widespread dissemination of valuable genotypes because of economics of semen use. In this technique, the semen is directly is deposited into the lumen of uterine horn and thus circumvents the cervical barrier and assures fertilization. Laparoscope aided intrauterine AI technique improves the pregnancy rate from 72.4 to 81.2% in Merino ewes inseminated with frozen semen (Ham et al., 2000). Gulph system of transcervical AI (Buckrell et al., 1992) is a simple, cost-effective and non-invasive technique. Trans-cervical AI technique in native sheep breeds have been developed at CSWRI, Avikanagar with the lambing rate from 22.7 to 38.2% (Naqvi et al., 1998, 1999). In the small ruminant the AI practices is not widely applied at the field level.

For the AI purpose, there should be research focused on developing protocol for preservation of semen. Because long term semen storage protocol is not available in sheep which will hinder AI practices in fields.

Multiple ovulation and embryo transfer: Multiple ovulation and embryo transfer (MOET) is a composite technology, which includes superovulation, fertilization, embryo recovery, *in vitro* culture of embryos, embryo freezing and embryo transfer. MOET offers large number of offspring produced by valuable females, increasing the population base of rare or endangered breeds or species and *ex situ* conservation of endangered populations. MOET improves the reproductive potential of female, in contrast to AI for male. It increases the female selection intensity and hence the rate of genetic improvement and it can be used to breed replacements from younger female thereby decreasing generation interval[25]. Multiple ovulation / superovulation is a process of harvesting multiple ova from an animal at a time. Superovulation can be induced by injecting fertility hormone in a female to grow more follicles to mature with increased ovulation. Several superovulatory protocols have been developed during the past decades using fertility hormones such as PMSG, FSH and GnRH in different combination in sheep. Uses of GnRH along with PMSG alone or PMSG plus FSH-P treatment increased ovulation rate two-three folds (Gulyani and Naqvi, 1996; Naqvi and Gulyani, 1998). It is concluded that MOET could produce substantial increase in the rate of genetic improvement in any species where natural reproductive efficiency is low. The rate of genetic improvement could even be doubled, but rate of inbreeding would also be substantially increased (Nicolus, 1996).

In vitro embryo production and embryo transfer: Embryo transfer technique can substantially improve the genetic makeup of animals in terms to increase meat, milk and wool production. The *in vitro* production of embryo is a necessary step to produce large number of embryos for embryo transfer, nuclear transfer and cloning experiments. Mature oocytes and capacitated sperms are pre-requisite for *in vitro* production of embryos and require optimum culture medium conditions for their development. Some of the limitations of MOET are the low average and high variability of embryo numbers. These limitations can be overcome by non-surgical harvesting of ova from females (called ovum pickup) and subsequently *in vitro* maturation (IVM) and *in vitro* fertilization (IVF), which can yield large number of transferable embryos. Collectively, these three procedures are termed as *in-vitro* embryo production (Nicolus et al., 1996). Methods for *in vitro* maturation of oocytes, fertilization and embryo culture have been used extensively in sheep *(*Walker,et al., 1992*).* In embryo collection, a surgical method is used in sheep and goat on day 3 or 4 following mating or AI. Recently, emphasis has been shifted from surgical to non-surgical laparoscope aided embryo collection technique either by direct or suction method (Anon, 2000). Mc Millan and Hall (1994) have developed a rapid and easy method of laparoscope aided embryo transfer in sheep. This technique has been modified and adopted for embryo transfer in native and crossbred sheep at CSWRI (Naqvi and Gulyani, 1995) Embryo transfer can be used much more efficiently if there is a method for embryo storage otherwise a large group of potential recipients must be available and these increase the cost very markedly.

Early pregnancy diagnosis: The purpose of early pregnancy diagnosis is to identify non-pregnant animals soon after mating or insemination so that time lost from infertility may be reduced. Early pregnancy diagnosis in sheep can be done through detection of early pregnancy factor (polypeptides) after a few days of conception. Polypeptides (i.e. antigens) specific to pregnancy have been reported in maternal tissues of various farm animal species. The antigens immunologically related to boPAG-1 (bovine pregnancy associated protein-1) and boPSP-B (bovine pregnancy specific protein-B) have been isolated from ovine fetal cotyledon i.e ovine PAG-1 (ovPAG-1) (Ziomek, 1998) and ovine PSP-B (ovPSP-B) (Willard et al., 1995). These ovine antigens have been detected in the plasma of pregnant ewes and all pregnant ewes were positive by 4th week (Ranilla et al., 1994). Ovine PSP-B was detectable from 21st day and concentration increased uniformly to 30th day (Willard et al., 1987). Pregnancy can be diagnosed by measuring progesterone concentration in the plasma on 16-18 days by radioimmunoassay (RIA). A major disadvantage of the RIA is the rigid safety standards-safety of handlers, user, and disposal of radioactive material. An

ultrasonography technique is used for early pregnancy diagnosis in sheep through imaging fluid in the uterine lumen for visualizing placentomes or identifying fetuses (Haibel et al., 1990). Accuracy with transabdominal scanning allows pregnancy diagnosis as early as 22nd day (Hessenlink et al., 1994). Transrectal imaging is more sensitive than transabdominal ultrasound and provides earlier pregnancy diagnosis, but requires a highly technical ability. At CSWRI, attempts have been made for diagnosis of early pregnancy by laparoscopy and pregnancy was diagnosed at 40th days after mating or insemination (Naqvi et al., 1999).

Embryo sexing: Sex of an individual is determined at the time of fertilization when union of ovum with X or Y bearing spermatozoa will culminate as female or male respectively. Sex of embryo can be determined by various methods such as Y- specific antigen, chromosome analysis, X-linked enzyme assay, Y-specific DNA probes and polymerase chain reaction (PCR) (Van Vliet et al., 1989). However, uses of male specific DNA probes specific to Y-chromosome accurately determined the sex of pre-implantation embryos. The probe can be either radiolabbled or non-radiolabeled DNA fragment, which is used to detect the presence of Y-chromosome in the blastocyst stage of embryo. The main disadvantage is that it is an invasive technique and decreases the viability of embryos. Recent developments in the recombinant DNA technology have made it possible to use PCR for amplification of Y-specific DNA sequence using special primers. The sex of ovine embryos was developed using PCR primers derived from ovine Y-chromosome randomly amplified polymorphic DNA markers. PCR assay has almost cent percent accuracy in confirming the sex of an individual (Gutierrez-Adan et al., 1997). The trophoblastic cells of ovine blastosyst were cultured in-vitro for different period and used them for embryo sexing. The sex of cells was assayed by PCR and 12 lambs were born after transfer of biopsied embryos confirmed its 100% accuracy (Leoni et al., 2000). Out of these techniques, PCR has been widely used for commercial embryo sexing in several countries like Finland, Germany, Hungry, Japan, UK and USA.

Sperm sexing: Sperm sexing is being used to separate the X and Y bearing spermatozoa from the semen. The most used technique for separation of X and Y chromosome bearing spermatozoa is flow cytometry and cell sorting. The high-speed cell sorter system is currently used for sorting viable sperm (Johnson and Welch, 1999; Johnson, 2000) and the accuracy of producing male or female by sorted sperm is 90% (Seidel et al., 1999). Fluorescence *in situ* hybridization is a technique in which fluorescent signal is counted to determine the proportion of Y-chromosome bearing sperm carrying Y-chromosome micro-satellite DNA probe (Kawarasaki et al., 1999). Quantitative PCR has been found to give useful information about the proportion of X and Y sperm in a sorted sample. It can be used effectively based on sorting single sperm in to 48 well plates and carrying out PCR reaction (Welch et al., 1997). Sort reanalysis for DNA has an advantage over FISH and PCR since PCR and FISH may take from 3 to 4 hrs (Fugger et al., 1998) while sort analysis can do within a 30 minutes. Sperm sexing is an indispensable technique in IVF, intrauterine insemination and embryo transfer programs for producing desired sex of offsprings.

Nuclear cloning: Nuclear cloning has brought revolution in reproductive technologies. Dolly was cloned from an udder cell of 6 year old Finn Dorset ewes is the first successfully cloned livestock (Campbell et al., 1996; Wilmut et al., 1997). It is reprogramming of cells and has the potential to produce a large numbers of genetically identical animals. Nuclear transfer offers several other possibilities of which production of transgenic animal will have a high impact on livestock industry. Nuclear transfer involves transferring the complete genetic material (the DNA contained in the nucleus) from one cell into unfertilized enucleated egg. Wells et al. (1998) cloned a sheep from cultured embryonic cell line in which a donar nucleus is transplanted in to enucleated MII oocyte. The reconstructed embryo is then cultured and transferred to surrogate mother for development to term (Polejaeva et al., 2000). Nuclear transplantation does not result in a genetically completely identical individual because the ovum donor contributes the mitochondrial genome. Although nuclear transfer has proved to be a valuable techniques but have several significant limitations. Only 1-2% of reconstructed embryos survive to become live offspring. Further research is essential to understand the mechanisms of reprogramming gene expression in the transferred embryos and the cause of failure. Applications of nuclear transfer in animal improvement programmes are not practicable because of its low efficiency (Dematawewa and

Berger, 1998) in particular the late pre and prenatal loss. However, there is the potential to significantly enhance genetic improvement, the dissemination elite germplasm from selected flocks in the future (Wilmut et al., 2000).

Transgenic technology

The transgenic animals are carriers of foreign genes into their genome. The DNA introduced is called 'transgene' and overall process is called transgenic technology or transgenesis. Microinjection is frequently used technique for transgenic production, where foreign DNA is transferred into pronuclei of fertilized ovum. This technique suffers from several serious limitations like DNA can only be added not deleted, the integration of foreign DNA is random into host genome and variable expression (Pursel and rexroad, 1993; Walls, 1996), time consuming and requires substantial intellectual and financial resources (Seidel, 1993). Other techniques have been developed for transgenesis viz. sperm mediated DNA transfer (Gandolfi, 1998; Squires et al., 1999), intracytoplasmic injection of transgenic sperm head (Perry et al., 1999) and somatic nuclear transfer. Transgenic animals show significant improvement in economically important traits such as growth rate, feed conversion efficiency and body fat muscle in farm animals and well documented in human medicine also. Several recombinant proteins have been produced in large amounts from a mammary gland of transgenic sheep and goat and purified from their milk such as human antithrombin III, tissue plasminogen activator, alpha-glucokinase and lectoferrin (Meade et al., 1999; Ziomek, 1998). Human cloning factor VIII cDNA construct can be expressed in the mammary gland of transgenic sheep (Niemann et al., 1999). A transgenic sheep called 'Tracy' produced human alpha -1- antitrypsin in their milk and now entered in clinical trials for cystic fibrosis. Polly, a cloned sheep carrying transgene for encoding factor IX, a protein involved in preventing haemophilia (Colman, 1999). Booroola fecundity (*FecB*) gene causes the high fertility in Australian Merino sheep. The mutation in this gene increases ovulation rate and litter size in Indian Garole and Kendrapada sheep. The *FecB* locus is situated in the region of bone morphogenetic protein receptor-IB (BMPR-1B). The BMPR-IB gene from carrier Garole sheep can be can be transfected / microinjected into an embryo or by suitable techniques for production of the transgenic animals. Likewise, the genes responsible for muscle growth (e.g. callipyge or myostatin) and parasitic resistance gene (MHC or interferon gamma if identified) will be utilized for production of the transgenic animals. The transgenic embryos will be transferred into recipient for its viable progeny and will be tested for prolificacy and other traits of economic importance. Gene farming will be a potential application of developing a transgenic sheep for treatment of the human diseases.

Animal genetics and breeding

Genetic improvement of livestock depends on access to genetic variation and effective methods for exploiting this variation. Genetic diversity constitutes a buffer against changes in the environment and is a key in selection and breeding for adaptability and production on a range of environments. A genetic or molecular marker for a trait is a DNA segment which is associated with, and hence segregates in a predictable pattern as, the trait. Genetic markers facilitate the "tagging" of individual genes or small chromosome segments containing genes which influence the trait of interest. Molecular markers opened many new vistas for animal genetic improvement such as determining genetic variability, marker assisted selection, disease resistance and genetic disorders in livestock species. Molecular markers may also play an important role in livestock improvement through selective breeding.

Genetic distance estimation: Genetic distance is a measurement of genetic similarity and dissimilarity between two populations (between species, breeds or strains) and serves as a useful tool for characterization of different breeds or strains within a species. Genetic distance can be measured on the basis of polymorphic characters occurring at different levels such as morphological, biochemical, cellular and DNA levels. Molecular markers are capable of generating individual specific DNA fingerprinting (DFP) patterns that is useful for establishing familial relationships (Jeffreys et al., 1986 and Jeffreys and Morton, 1987). RAPD-PCR fingerprinting has been used for measuring genetic distance in cattle and sheep (Gwasika, et al., 1994; Kantanen et al., 1995).

Parentage testing: Parentage testing using molecular markers yields much higher probability than the testing with blood groups or other biochemical markers (Geldermann, 1990). Highly polymorphic DNA fingerprinting markers are more useful for parentage testing in livestock species. DNA fingerprinting with oligoprobes (OAT18 and ONS1) has been successfully used for determining the parentage of IVF buffalo calf (Mattapallil and Ali, 1988). PCR based microsatellite assays have been reported for parentage testing in different livestock species for e.g. in cattle (Glowatzki-Mulis et al., 1995). Molecular markers serve as a tool for animal identification particularly for verification of semen used in artificial insemination.

Marker assisted selection (MAS): Molecular markers may help in selecting the animals for enhancement of production traits for which information is available. The selection of animals can be done right at birth since identification of potential genotypes does not need expression of the trait in the individual if molecular markers known. The application of MAS within population is the selection of young sires before their induction for actual progeny testing (Kashi et al., 1990; Weller and Fernando, 1991)and this may lead to an increase of genetic gain by 15-30% (Weller and Fernando, 1991). Presently work is being carried out at different laboratories to find out markers related with different production, survivability and disease resistant traits. They may help the traditional breeding practices to enhance their applicability multi-folds. *FecB* is a very good example of marker assisted selection. The Booroola fecundity gene (*FecB*) is the first identified gene for high fecundity in sheep, and it is an autosomal mutation. The *FecB* gene increases ovulation rate and litter size in sheep (co- dominant for ovulation and partially dominant for litter size). Moreover, introgression of *FecB* gene could improve the fecundities of low prolific flocks (Hua et al., 2009). Thus, *FecB* introgression programmes have been initiated in several countries and the results of these programs have improved the prolificacy. The information of the meat quality trait loci can be applied in breeding programs by using marker-assisted selection (MAS). Using different DNA marker technologies, several genes affecting meat quality have been identified. For example, the Halothane gene, the Rendment Napole (RN) gene and insulin-like growth factor-2 (IGF2) gene in pig, the calpastatin and the calpain one gene in bovine, callipyge phenotypes in sheep, etc. The molecular basis of meat quality is being revealed by functional genomics approaches. Research is currently underway to identify genetic markers for wool, milk, growth and other reproduction related genes in Indian sheep population.

Identification of genetic disorders: Molecular markers in addition to their potential role in animal breeding will also enable to test the precious animals for risk of genetic disorders. Many of the inheritable diseases may not result from the infection with bacteria or virus, but the defect in the genome of the individual host. Sometimes certain allelic variation in the host genome may lead to susceptibility or resistance to a particular disease. Several genetic disorders may be caused by a single mutation for e.g. Citrulinaemia (Dennis et al., 1989), bovine leukocyte adhesion deficiency (Shuster et al., 1992) and uridine monophosphate synthatase (Schwenger et al., 1994) in cattle possessing the defective recessive allele can be identified by PCR-RFLP technique. Molecular markers may help to identify affected/carrier animals at an early stage of life; thereby breeding can be avoided for these animals carrying genotypes for genetic disorders.

Breeding for disease resistance

Despite traditional control measures, losses to infectious diseases continue to impede the livestock industries. An alternative approach to this problem is the exploitation of genetic disease resistance involving both immune and non-immune mechanism, which is the inherent capacity of animal to resist disease. Molecular marker can play an important role in identifying genotypes resistant to several diseases. Marker linked with gene conferring resistance against disease(s) or within the gene will help in defining strategies for development of disease resistant livestock. Research is currently underway to identify genetic markers for resistance and susceptibility of sheep against *Haemonchus contortus* parasite in India. Molecular analysis of pathogens (bacterial and viral) causing disease in sheep needs to be investigated by PCR and real time PCR.

Rumen biotechnology

Biotechnological options are available for improving rumen fermentation and enhancing the nutritive value and utilization of agro-industrial by products and other forages. Fibrous feeds, including crop residues, of low digestibility constitute the major proportion of feed available to the ruminants. The associated low productivity can be overcome by balancing of nutrients for the growth of rumen microflora thereby facilitating efficient fermentative digestion and providing small quantities of bypass nutrients to balance the products of fermentative digestion, enhancing digestibility of fibrous feeds through treatment with alkali or by manipulating the balance of organism in the rumen and genetic manipulation of rumen microorganisms. Rumen microorganisms 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. 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.

Conclusions

It is concluded that a several reproductive biotechnology tools have been developed over the past decades that substantially improve the reproductive efficiency and for genetic improvement in the livestock sector. Further research efforts are needed to develop less invasive techniques for non-surgical collection of embryos in sheep. Sperm sexing technology which sort sperm into X and Y population at 90% of purity that is adaptable would have more impact on artificial insemination programmes. The utilization of nuclear transfer technique in livestock species provides enormous benefits in the livestock industry in terms of increasing growth rate, fecundity and feed conversion efficiency. The incorporation of fecundity gene in to sheep genome may be more useful for improving the prolificacy of low prolific sheep and offers the possibility to increase the number of offspring. Transgenic sheep produces a large amount of pharmaceutical proteins and growth factors would be more benefit in human mankind for curing of several diseases. However the efficiency of procedure is still low in relation to pregnancy and development to term rates, which are significantly lower than in-vitro produced embryos. There is an urgent need to understand molecular mechanisms and gene expression controls of mammalian development.

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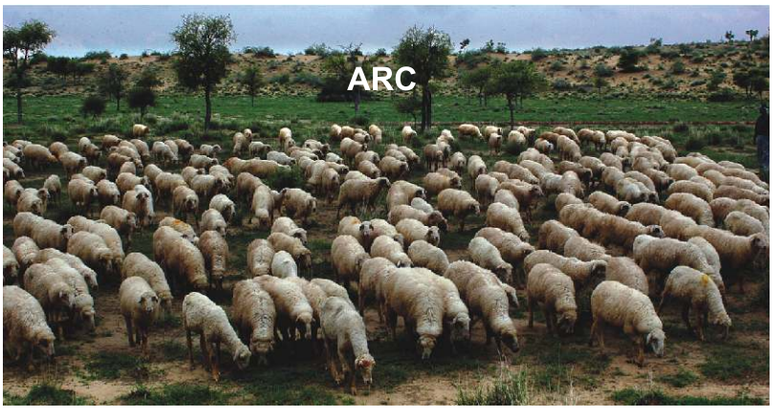
Important Milestones of the Institute

1962 -	Establishment of institute at Avikanagar
1963 -	Establishment of NTRS, Garsa, Kullu
1964 -	Introduced Romney Marsh, South Down and Rambouillet sheep at Avikanagar
1965 -	Establishment of SRRC, Mannavanur
1967 -	Constructed office-cum- laboratory building
1968 -	Wet processing and spinning plant
1969 -	Constructed post graduate hostel building
	Introduced Corriedale sheep at SRRC, Mannavanur
1970 -	Constructed medical dispensary at Avikanagar
1971 -	Introduced Soviet Merino sheep at Avikanagar
1972 -	Constructed animal health laboratory
1974 -	Establishment of ARC, Bikaner
	Introduced Dorset and Suffolk sheep at Avikanagar
1975 -	Introduced Karakul sheep at Bikaner
1977 -	Evolved Avikalin and Avivastra sheep
1978 -	Introduced rabbits at NTRS Garsa
1981 -	Constructed new administrative building
1982 -	Introduced rabbits at CSWRI, Avikanagar and SRRC, Mannavanur
1983 -	Evolved synthetic strains of Mutton, Nali and Chokla
1985 -	Constructed NPB building
1986 -	Developed disease data information system for organized sheep farm
	Evolved Bharat Merino sheep
1988 -	Constructed administration- cum -laboratory building at Bikaner
1989 -	Constructed model rural slaughter house and rabbit sheds at Avikanagar
	Implemented planned flock health calendar
1990 -	Lambs born using pelleted frozen semen
1991 -	Developed protocal for freezing of ram semen in straws
1992 -	Lambs born through embryo transfer technology
	Established Asian Small Ruminant Information Centre (ASRIC)
1995 -	Constructed central school building at Avikanagar and office-cum-guest house at Jaipur
1996 -	Introduction of Awassi sheep at Avikanagar
1997 -	Introduction of Garole sheep at Avikanagar
	Transferred Goat Unit of WRRC, CIRG to CSWRI, Avikanagar
	Established VSAT facilities at Avikanagar
1998 -	Developed protocol for cryopreservation of embryos
	Implemented one anthelmintic drench per annum in sheep at Avikanagar
2002 -	Complete feed block for feeding during scarcity
	Developed lamb feeding protocol
2003 -	Constructed guest house at Avikanagar
2004 -	Recovered 24 embryoes in single flushing in Garole sheep
	Implemented region specific worm management programme for sheep flocks in Rajasthan
2007 -	Constructed biotechnology building
	Constructed auditorium
	Developed FROGIN
	Developed area specific mineral mixture
	Impregnated intra vaginal sponges for estrus synchronization
2009 -	Introduced Patanwadi sheep at Avikanagar
	Established model micro watershed management system
2010 -	Introduced Kendrapada sheep at Avikanagar
2011 -	Constructed Agricultural Technology Information Centre at Avikanagar

Five Decades of Service to Indian Sheep Husbandry



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