NUTRACEUTICALS IN LIVESTOCK & POULTRY

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1 Introduction

The term “nutraceutical” was coined from “nutrition” and “pharmaceutical” in 1989 by Stephen DeFelice, MD, founder and chairman of the Foundation for Innovation in Medicine (FIM), Cranford, NJ. According to DeFelice, nutraceutical can be defined as, “a food (or part of a food) that provides medical or health benefits, including the prevention and/or treatment of a disease”. In other words, nutraceuticals are chemical or natural feed ingredients which may enhance health by providing a physiological benefit by the provision of basic nutrients. Enzymes, prebiotics, probiotics, yeast and fungal extracts, predigested hydrolyzed carbohydrates and fats, phytogenic additives, acids etc are included in nutraceuticals. However, the term nutraceutical as commonly used in marketing has no regulatory definition.

A nutraceutical is a product isolated or purified from foods that is generally sold in medicinal forms not usually associated with food. A nutraceutical is demonstrated to have physiological benefit or provide protection against chronic disease. On the other hand, “functional feed” is similar in appearance to a conventional food that is consumed as a part of usual diet, and is demonstrated to have physiological benefits to reduce the risk of chronic disease beyond basic nutritional function, i.e. they contain bioactive compound. The different types of nutraceuticals have been classified in Table 1.1. However, only few of them have been studied in detail pertaining to their specific mode of action in different species, synergistic action etc. Studies on nutraceuticals have gained importance after the ban on sub-therapeutic use of antibiotics in several countries in the last century. It is expected that the trend will continue in the present century too along with a search for newer nutraceuticals for optimum production in livestock and poultry.

Table 1.1. Different types of nutraceuticals used in livestock, poultry and pig

Nutraceutical Type	Examples	Expected Actions	Research Support/Status
Antioxidants	Vitamin E & Selenium; Vitamin C; Grape seed extract; lipoic acid etc.	Minimize oxidative damage to reactive oxygen species generated during intense exercise or inflammatory conditions.	A significant number of studies have been published addressing various aspects of antioxidant effects. Study results have been mixed, but most suggested of some positive effect.
Antimicrobials	Antibiotics; Anthelmintics etc.	Provide therapy or prevention of specific pathogen associated diseases and act as growth promoter.	Highly regulated by FDA and must be used accordingly.

Herbal additives	Wide range of whole plants or Extracts.	Anti-methanogenic antiinflammatory; metabolic effects.	Lot of studies have been conducted recently on their effect as rumen fermentation modulator.
Enzymes	Phytase; Cellulase; Hemicellulase	Improve digestive capacity of specific compounds, nutrients within consumed diet	Fiber digestibility is reported to be increased with enzymes.
Ergogenic Agents	Chromium; Creatine; Betaine (TMG); Dimethyl-glycine (DMG)	Improve muscle mass and function. Increase muscle capacity for aerobic metabolism and facilitate recovery	A limited number of studies have been published on various agents in case of pigs. Most have shown no effect of supplement compared to controls.
Oligosaccharides	Fructooligosaccharides; mannan-oligosaccharides etc.	Alter intestinal microbial populations by either stimulating growth of beneficial bacteria or inhibiting pathogens	Limited studies published. Potential for use in pigs and calves. But needs further study.
Organic Minerals	Chelates; Proteinates;Complexes; Polysaccharides	Improve bioavailability of mineral elements from the diet	Studies varied in type of organic source versus inorganic source. Limited effects were seen with organic mineral supplementation and mostly with younger animals.
Omega-3 Fatty Acids	EPA, DHA, Flaxseed, Fishoils	Anti-inflammatory properties, counter regulatory	Studies showed alteration in either plasma or membrane fatty acids profile based on source. Most studies showed anti-inflammatory

		metabolically to n-6 fatty acid derived eicosanoids compounds	effects with n-3 fatty acids. Clinical studies suggest reduced disease skin allergies with n-3 supplementation.
Organic acids	Acetic acid, Propionic acid, Butyric acid, citric acid	It reduces intracellular pH and thereby reduces enzyme activity leading to death of bacteria	Studies on various organic acids conducted in ruminants, pigs and poultry
Probiotics	Various lactic acid producing bacteria (live cultures).	Alter ruminal fermentation pattern. Alter intestinal microbial populations by providing live beneficial bacteria that inhibit pathogen growth.	Mixed results reported and suggested beneficial effect as rumen modulator.
Yeast and Yeastextracts	Live or culture extracts *Saccharomyces cerevisiea*	Improve fiber digestibility and dietary nutrient availability; Alter intestinal microbial flora.	Most studies showed some improvement in nutrient availability. Some studies have documented improved performance.

2 Antibiotics

Feed accounts for a major portion of farm expenses in livestock and poultry production. Thus, to obtain maximum profitability, it must be ensured that the feed should not only be well balanced nutritionally but economical too. Rising prices of feeds certainly have reduced the profitable nature of broiler farming. For better utilization of feed and to improve feed efficiency, antibiotics were used at sub-therapeutic levels in the animal ration.

Development of antibacterials progressed very quickly and as a result, certain new antibacterial substances were discovered and developed for use as growth promoting agents in livestock and poultry. The antibiotics act by reducing nutrient destruction in the intestine, increasing microbial synthesis of certain vitamins and amino acids, reducing the rate of passage of nutrients in the gastrointestinal tract and reducing the thickness of the intestinal wall. This thinner wall and reduced rate of passage have been suggested as factors favoring efficient absorption of nutrients. It is also reported that the antibiotics also inhibit the bacterial production of ammonia and other harmful nitrogenous compounds such as trimethylamine that reduce the growth of chickens.

Poultry

Antibiotics have been used at sub-therapeutic levels for promoting the growth and immunity of birds. The advantages of using antibiotics as feed supplements in terms of growth stimulation and improvement of feed efficiency have been well documented (Ensminger *et al*., 1990; Peterson *et al*., 1991).

Antibiotic as growth promoter in poultry feeds

George *et al*. (1982) noted that birds fed virginiamycin had significantly less mortality and lower intestinal lesion scores compared to non-medicated birds when experimentally infected with necrotic enteritis. Abou *et al*. (1983) reported that virginiamycin administered to the experimentally infected group for 8 weeks in the feed of broilers at a concentration of 25 g/ton had no beneficial effect compared to an infected control group not receiving the antibiotic.

Body weight and bone ash at 21-days of age were improved by the addition of virginiamycin in the diet of broilers. Also, the amount of phosphorus required to produce a gram of body weight was decreased by the addition of virginiamycin in the diet (Buresh *et al*., 1985).

Bartov *et al*. (1992) reported that virginiamycin had no significant effect on food intake or weight gain, but significantly improved food efficiency up to 28 days of age in broiler chicks. Further, virginiamycin significantly decreased fat excretion and improved fat relative retention. Similarly, Al-Batshan *et al*. (1992) noted that virginiamycin improved body weight and feed efficiency from 1 to 29 days of age in turkey poults, irrespective of type of litter or disease condition such as stunting syndrome.

Proudfoot *et al*. (1990) noted that virginiamycin supplementation had no significant effect on mortality or feed conversion ratios in male chicken broilers, regardless of the mode of administration. However, body weights at 21 days of age but not at 42 days of age were significantly heavier for broilers receiving virginiamycin via the drinking water. The inclusion of virginiamycin in the feed failed to improve body weights at either 21 or 42 days of age. Miles *et al*. (1984) reported that diet supplemented with virginiamycin resulted in an increase in xanthophyll utilization and feed efficiency was significantly improved by the supplementation of 5 and 10 ppm of virginiamycin in the broiler diet. Salmon *et al*. (1990) noted that the combination of virginiamycin and monensin resulted in superior overall feed efficiency to that obtained with either additive alone in turkey broilers.

Tokosova *et al*. (1990) studied the effect of cyadox and virginiamycin on Marek's disease in chickens. The weight gains on the 56th day and dressing percentage were highest, in comparison to the control group, in the virginiamycin-treated group. Decrease in heart weight occurred in the cyadox treated group where as increase in liver and heart weight and intestine shortening occurred in the

virginiamycin treated group as compared to the control group. Woodward *et al*. (1988) noted that the body weights were higher at higher energy levels with addition of virginiamycin to the diets. Further, yield was increased from 63.3 to 64.0% by dietary virginiamycin.

Henry *et al*. (1986) reported that the virginiamycin decreased relative intestinal tract weight and increased kidney and bone manganese indicating that virginiamycin increased absorption of manganese.

Ruminants

The ruminant animal does not efficiently convert feedstuffs into meat or milk. As a result, several strategies have been used to improve ruminant feed efficiency. One of the techniques to improve the efficiency of the fermentation has included the addition of antimicrobial compounds in the diet to alter the ruminal microbial ecosystem. Ionophores, antibiotics e.g. monensin, lasalocid, laidlomycin, salinomycin, narasin etc. and conventional antibiotics e.g. chlortetracycline, oxytetracycline, bacitracin, tylosin etc. are antimicrobial compounds that are commonly fed to ruminant animals to improve feed efficiency.

Ionophores are antibiotic class that alters rumen fermentation characteristics. Ionophores cause cattle to grow more efficiently (Russell and Strobel, 1989) but were originally used to control intestinal parasites (coccidiostat) in poultry (Bergen and Bates, 1984). Monensin has been marketed for cattle as a methane inhibitor and propionate (the most efficiently utilized gluconeogenic VFA) enhancer (Dinius *et al*., 1976; Richardson *et al*., 1976; Russell and Strobel, 1989). Additional benefits of monensin usage include a reduction of dietary protein deamination, resulting in less ammonia urinary excretion (Russell and Strobel, 1989) and a decrease in lactic acid production (Dennis *et al*., 1981) which results in a reduction in ruminal acidosis (Russell and Strobel, 1989) and liver abscesses (Nagaraja and Chengappa, 1998). The increases in energy availability and nitrogen retention improve the efficiency of feed utilization by the ruminant animal, and thus improve animal productivity and production profitability (Potter *et al*., 1976; Russell and Strobel, 1989). Monensin treatment reduces morbidity and mortality among feedlot animals by reducing the incidence of acute and sub-acute ruminal acidosis, bloat, and bovine emphysema (Galyean and Owens, 1988). Dietary carbohydrates are rapidly fermented in the rumen which can result in an accumulation of lactic acid, resulting in a lowered ruminal pH, ruminal dysfunction and acidosis (Nagaraja *et al*., 1982; Burrin and Britton, 1986; Russell and Rychlik, 2001). Ruminal acidosis is associated with reduced feed intake, lowered feed efficiency and cyclic feeding, as well as the death of some animals. Monensin reduces acidosis by directly inhibiting the lactate-producing bacteria (e.g., Streptococcus bovis, lactobacilli) (Dennis *et al*., 1981).

All improvements in animal productivity caused by ionophore treatment represent a secondary effect caused by the disruption of normal bacterial membrane physiology (Bergen and Bates, 1984). Ionophores are moderate molecular weight compounds (~700 MW) that are mobile ion carriers (Pressman, 1976). Because ionophores are highly lipophilic, they rapidly dissolve into bacterial cell membranes (Pressman, 1976). Ionophores bind ions, shield the ionic charges and translocate ions across the bacterial membrane, disrupting crucial ion gradients (Pressman, 1976). Because ionophores are lipophilic compounds that exert their effects at the membrane level, they are most effective against gram-positive bacteria. The peptidoglycan layer that surrounds gram-positive bacteria is porous, and allows small molecules to pass through, reaching the cytoplasmic membrane where the lipophilic ionophore rapidly dissolves into the membrane. Conversely, gram-negative bacteria, are separated from the environment and antimicrobial agents by a lipopolysaccharide layer, outer membrane and periplasmic space. Monensin is bound by both grampositive and gram-negative bacteria (Chow *et al*., 1994).

Ionophores are potent antimicrobial compounds that improve production efficiency and health in cattle by altering the composition of the ruminal microbial ecosystem, thereby reducing the incidence of illnesses related to the ruminal fermentation (e.g., bloat, bovine emphysema). However, like all other antimicrobial compounds, concerns have been raised about the development of antimicrobial resistance and the potential for the transfer of cross resistance to antibiotics used in human medicine.

Conventional antibiotics have been used in animal feed for about 50 years ever since the discovery not only as an anti-microbial agent, but also as a growth-promoting agent and improvement in performance. Tetracyclines, penicillin, streptomycin and bactrican soon began to be common additives in feed for livestock. Currently, the following antibiotics are used in livestock: chlortetracycline, procaine penicillin, oxytetracycline, tylosin, bacitracin, neomycin sulfate, streptomycin, erythromycin, linomycin, oleandomycin, virginiamycin and bambermycins. In addition to these antibiotics, which are of microbial origin, there are other chemically synthesized antimicrobial agents that are also sometimes used in animal feeds. These include three major classes of compounds: arsenical, nito-furan and sulfa compounds. Arsenical compounds include arsanilic acid, 3-nitro-4-hydroxy phenylarsonic acid and sodium arsanilate; nitro-furan compounds include furazolidone and nitro-furazone; sulpha compounds include sulfamethazine, sulfathiazole, and sulfaquinoxaline. Antibiotics are used regularly in animal feed in many countries at a rate of 2 to 50 grams per ton for improved performance in the animals (McDonald *et al*., 1997). The reasons include a more efficient conversion of feed to animal products, an increased growth rate and a lower morbidity/mortality rate in general. The levels of antibiotics are often increased to 50-200 grams/ton or more when specific diseases are being targeted as when the spread of a particular disease is rampant. The levels are also increased in times of stress. This increased amount is often decreased when the threat of a disease is gone.

After animals have been fed antibiotics over a period of time, they retain the strains of bacteria which are resistant to antibiotics

(FDA, 1995). These bacteria proliferate in the animal. Through interaction, the resistant bacteria are transmitted to the other animals, thus forming a colonization of antibiotic resistant bacteria. The bacteria flourish in the intestinal flora of the animal, as well as, in the muscle. As a result, the feces of the animal often contain the resistant bacteria. Transfer of the bacteria from animal to human is possible through many practices. The primary exposure of humans to resistant bacteria occurs in farms and slaughterhouses.

The possibility of developing resistant population of bacteria and side effects of using antibiotics as growth promoters in farm animals have been of immense concern, principally with regard to the loss of efficacy of antibiotic as growth stimulant and for controlling an outbreak of bacterial disease (Hinton, 1988). Some early studies showed that continuous feeding of antibiotics to chickens resulted in a decrease growth response. The possible emergence of antibiotic-resistant strains of bacteria on the premises where growing birds are fed on low concentration of antibiotics has been reported (Narayanankutty *et al*., 1992). Following the discovery of resistance transfer factor, there has been growing concern about public health risk resulting from antibiotic resistance, carcinogenic responses and other side effects of residues in food. The most important potential route by which humans become infected with resistant bacteria is through meat, milk and eggs (Hinton, 1988). As poultry meat is one of the most important sources of animal protein throughout the world, even a low incidence of cross infection could be important. The possibility of developing resistant bacteria besides other side effects when antibiotics are used as growth promoters have led to the recent EU ban on the use of most antibiotics on farm animals as growth promoters. This ought to have serious consequences in growth performance of poultry. Therefore, an intensive search for alternative feed additives started in the last decade.

References

Abou Youssef, M. H., Di Cuollo, C. J., Free, S. M. and Scott, G. C. 1983. The influence of a feed additive level of virginiamycin on the course of an experimentally induced *Salmonella typhimurium* infection in broilers. *Poultry Sci*ence, **62**: 30-37.

Al-Batshan, H. A., Sell, J. L., Piquer, J., Mallarino, E., Soto-Salanova, M.F. and Angel, C.R. 1992. Responses of turkey poults to virginiamycin as influenced by litter condition and experimentally induced stunting syndrome. *Poultry Science*, **71**: 894-904.

Bartov, I. 1992. Lack of effect of dietary energy-to-protein ratio and energy concentration on the response of broiler chickens to virginiamycin. *British Poultry Science*, **33**: 381-391.

Bergen, W.G., and Bates, D.B. 1984. Ionophores: Their effect on production efficiency and mode of action. *Journal of Animal Science*, **58**: 1465-1483.

Buresh, R. E., Miles, R. D. and Harms, R. H. 1985. Influence of virginiamycin on phosphorus utilization by broiler chicks. *Poultry Science*, **64**: 757-758.

Burrin, D.G. and Britton, R.A. 1986. Response to monensin in cattle during subacute acidosis in cattle. *Journal of Animal Science*, **63**: 888-893.

Chow, J.M., Van Kessel, J.A.S. and Russell, J.B. 1994. Binding of radiolabeled monensin and lasalocid to ruminal microorganisms and feed. *Journal of Animal Science*, **72**: 1630-1635.

Dennis, S.M., Nagaraja, T.G. and Bartley, E.E. 1981. Effects of lasalocid or monensin on lactate-producing or -using rumen bacteria. *Journal of Animal Science*, **52**: 418-426.

Dinius, D.A., Simpson, M.E. and Marsh, P.B. 1976. Effect of monensin fed with forage on digestion and the ruminal ecosystem of steers. *Journal of Animal Science*, **42**: 229-234.

Ensminger, M. E., Oldfield, J. E., and Heinemann, W. 1990. *Feeds and Nutrition*. Ensminger Publishing Company, USA.

FDA. 1995. The Rise of Antibiotic-Resistant Infections. *Consumer*, 29. http://www.fda.gov/fdac/features/795_antibiotic.html

Galyean, M.L., and Owens, F.N. 1988. Effects of monensin on growth, reproduction, and lactation in ruminants. In: ISI Atlas of Science: *Animal and Plant Science*, ed. ISI Press, Philadelphia, PA. p. 71-75.

George, B. A., Quarles, C. L. and Fagerberg, D. J. 1982. Virginiamycin effects on controlling necrotic enteritis infection in chickens. *Poultry Science*, **61**: 447-450.

Henry, P. R., Ammerman, C. B. and Miles, R. D. 1986. Influence of virginiamycin and dietary manganese on performance, manganese utilization, and intestinal tract weight of broilers. *Poultry Science*, **65**: 321-324.

Hinton, M. H. 1988. Antibiotics, poultry production and public health. *World's Poultry Science Journal*, **44**: 67-69.

McDonald, L. C., Matthew J. K., Fred C. T. and William R. J. 1997. Vancomycin-Resistant Enterococci Outside the Health-Care Setting: Prevalence, Sources, and Public Health Implications. *Centers of Disease Control and Prevention, Alanta, Georgia*, **3**. wysiwyg://67/http://www.cdc.gov/ncidod/EID/vol3no3/mcdonald.htm

Miles, R. D., Janky, D. M. and Harms, R. H. 1984. Virginiamycin and broiler performance. *Poultry Science*, **63**: 1218-1221.

Nagaraja, T.G. and Chengappa, M.M. 1998. Liver abscesses in feedlot cattle: A review. *Journal of Animal Science*, **76**: 287-298.

Nagaraja, T.G., Avery, T.B., Bartley, E.E., Roof, S.K. and Dayton, A.D. 1982. Effect of lasalocid, monensin or thiopeptin on lactic acidosis in cattle. *Journal of Animal Science*, **54**: 649-658.

Narayanankutty, K., Ramakrishnan, A. and Viswannath, A. 1992. Efficacy of virginiamycin as growth promoter in commercial

broiler chicks. *Journal of Veterinary and Animal Sciences*, **23**: 96-97.

Peterson, R. A., Carpenter, G. H. and Jones, W. T. 1991. Effect of tetracycline hydrochloride and oxytetracycline hydrochloride given via drinking water on early mortality of broiler chicks from twenty- eight-week-old dams. *Poultry Science*, **70**: 1040-1042.

Potter, E.L., Raun, A.P., Cooley, C.O., Rathmacher, R.P. and Richardson, L.F. 1976. Effect of monensin on carcass characteristics, carcass composition and efficiency of converting feed to carcass. *Journal of Animal Science*, **43**: 678-683.

Pressman. 1976. Biological applications of ionophores. *Annual Review of Biochemistry*, **45**: 501-530.

Proudfoot, F. G., Jackson, E. D. and Hulan, H. W. 1990. The response of male chicken broilers to the dietary addition of virginiamycin. *Poultry Science*, **69**: 1713-1717.

Richardson, L.F., Raun, A.P., Potter, E.L., Cooley, C.O. and Rathmacher, R.P. 1976. Effect of monensin on rumen fermentation in vitro and *in vivo*. *Journal of Animal Science*, **43**: 657- 664.

Russell, J.B. and Rychlik, J.L. 2001. Factors that alter rumen microbial ecology. *Science*, **292**: 1119-1122.

Russell, J.B. and Strobel, H.J. 1989. Effect of ionophores on ruminal fermentation. *Applied and Environmental Microbiology*, **55**: 1-6.

Salmon, R. E. and Stevens, V. I. 1990. Virginiamycin and monensin, alone or in combination, in turkey broiler diets. *Poultry Science*, **69**: 1016-1019.

Tokosova, M. 1990. Observations of the effects of cyadox and virginiamycin on the course of Marek's disease in chickens. *Veterinarni Medicina*, **35**: 105-112.

Woodward, S. A., Harms, R. H., Miles, R. D., Janky, D. M. and Ruiz, N. 1988. Influence of Virginiamycin on yield of broilers fed four levels of energy. *Poultry Science*, **67**: 1222-1224.

3 Probiotics

The increasing use of antibiotics in livestock and poultry led to development of antibiotic resistant micro organisms and antibiotic residues in livestock products besides other side effects. These caused alarm bells ringing all over the world and by the end of June, 1999 majority of antibiotics as growth promoters in monogastric diets had been banned within the EU. The ban on use of antibiotic at sub therapeutic level in Europe and the potential for a ban in the United States have made scientists to seriously think of alternatives to antibiotics. In fact, the use of probiotics for farm animals was already stimulated by the findings of the Schwann Committee in 1969 which recommended that antibiotics in animal feeds should be restricted. However, the concept of probiotics gained momentum in later part of the last century. In recent years, with the public disapproval of using antibiotics in livestock and poultry production due to their residual effects in their products, there has been a greater emphasis on the use of probiotics in poultry.

'Probiotic' is a word derived from Greek word 'pro' and 'biotic' meaning 'for life'. Probiotics can be described as organisms and substances which contribute to intestinal microbial balance (Sperti, 1971). Fuller (1989) revised the definition as a live microbial feed supplement, which beneficially affects the host animal by improving the intestinal microbial balance.

The term probiotic was coined by Parker (1974) to describe organisms and substances, which contribute to intestinal microbial balance. However, the use of probiotic was first suggested by Elias Metchnikoff (1907) as he realized the role of probiotics in combating various disorders of gastrointestinal tract. He explained that the detrimental microbes in the intestinal tract excreted substances that were harmful to the host, through the constant infusion of 'friendly' organisms in the diet. Colonization of the gastrointestinal tract by disease causing (pathogenic) unfriendly organism was prevented and thus health and life expectancy improved. Thus the concept of microbial inoculation, based on the principle of 'competitive exclusion' was established.

Marriott and Davidson (1923) reported that young infant fed similar quantities of fresh cow milk or milk to which lactic acid was added had higher mean daily weight gains. Since then, the different types of probiotics have been tested like lactic acid producing bacteria, live yeast culture etc. In fact, nearly all the probiotics available in the market today contain *Saccharomyces cereviscae*, bacteria or their combination.

Probiotic may be classified into two major types: viable microbial cultures viz. Lactobacilli, Streptococci, Bacillus etc and microbial fermentation products. Basically a delicate balance exists between beneficial and healthy bacteria in a healthy bird and an imbalance leads to dysbacteriosis. Optimum growth of both beneficial and pathogenic micro organism is controlled by pH of intestine. This imbalance may be caused due to various factors like contaminated feed and water, environmental stress, overcrowding, prolonged use of antibiotics, infection etc. Under these changed circumstances, the putrefactive bacteria predominate and impair the normal gut function. Among all the species of bacteria that help in maintaining the normal gut function, Lactobacilli species is most affected under the changed circumstances and ultimately fail to maintain proper pH (4.5).

Several mechanisms have been postulated to explain the mode of action of probiotics. They are as follows:

- Probiotics cause beneficial changes in the gut flora with reduction in population of E.coli. Lactobacilli, the most common microbe of probiotic compete with coliforms and pathogenic microorganisms for the site of adherence on the intestinal surface.
- Probiotics like lactobacillus increase lactic acid production with subsequent change in intestinal pH. Lactic acid release in the intestine reduces the pH up to 4.5, which is lethal for pathogens but allow the growth of some strains of Lactobacilli.
- Probiotics produce antibiotic type substances. Live yeast culture is capable of releasing phytase for phosphorous digestion, lipase for fat digestion and endotryptase for protein digestion. It triggers the growth of cellulolytic bacteria resulting into digestion of fibre portion of feed. The yeast also facilitates the growth of lactobacillus. Yeast ferments the carbohydrate. The fermentation products inactivate the intestinal toxins that reduce the appetite of poultry. Yeasts

are also a rich source of vitamin B complex.
- Probiotics reduce the release of toxins probably with suppression of E.coli.

Characteristics of an ideal probiotic

- It should be capable to survive and metabolize in the gut environment i.e. it should be resistant to low pH.
- It should multiply faster in the digestive tract.
- It should be capable of exerting a beneficial effect on the host animal i.e. elicit growth and production.
- It should not be pathogenic or toxic to the host.
- The adhesive capacity of the probiotics should be firm and faster.

Poultry

Jin *et al*. (1997) concluded that the mode of action of probiotics in poultry includes (1) maintaining normal intestinal micro flora by competitive exclusion and antagonism. (2) Altering metabolism by increasing digestive enzyme activity and decreasing bacterial enzyme activity.

Use of probiotics as growth promoter

Fuller (1995) reported beneficial effects on growth rate, feed conversion efficiency and resistance to disease in broilers fed probiotics. Since then, different types of probiotics have been used singly or in combination to improve growth and FCR in different species of poultry.

Effect of probiotics on gut micro flora

Probiotics help in maintaining or restoring a stable micro flora in the gut of chickens that has been affected by pathogens and growth depressing bacteria (Barrow, 1992). Thus, use of probiotics based on specially selected bacteria have been suggested to normalize the intestinal micro flora and increase resistance to physical and physiological stress in intensively reared chickens (Tibiletti, 1993). It has been seen that microbial combination of different strains of *Lactobacilli, Streptococci* and *Sacchromyces cerevisae* from indigenous sources fed up to the grower phase were well established in the gastro intestinal tract of birds, eliciting beneficial effect throughout the laying period (Sharma *et al*., 2001).

Health and production performance

It has been noted that dietary supplementation of probiotics in broilers resulted in a significant reduction on the total serum cholesterol concentration and crop *Escherichia coli* count (Panda *et al*., 2001). Further, health and production traits of broilers are positively influenced and improved by feeding probiotics. Addition of probiotic to broiler diets reduce mortality rate due to ascites (Kahraman *et al*., 1997).

Use of probiotics in layer diets result in better hen body weight, egg production, egg weight, egg mass, egg component percentages, egg shell thickness, egg shell weight, feed intake, feed conversion, livability, decrease in meat & blood spots and decreased egg yolk and serum cholesterol (Huthail, 1996; Yakout *et al*., 2004 and Kurtoglu *et al*., 2004).

Use of probiotic in chicken diet results in reduced abdominal fat content, improved fatty acid digestibility, increased mineral utilization including organic phosphorus utilization, increased tibial ash and enhanced feed efficiency.

Use of probiotic in poultry diets may elicit the immunocompetence traits in poultry (Panda *et al*., 2008 & Dalloul *et al*., 2003).

Carcass quality characteristics of the broilers

Supplementation of probiotics in broiler diets may result in significant improvement in gain, feathering, dressing and edible meat yields without any undesirable change in the chemical composition and organoleptic quality of broiler meat (Krueger *et al*., 1990, Yalcin *et al*., 1993 & Banday and Risam, 2001).

Ruminants and pigs

Before birth, developing animals are sterile in the womb of their mothers. Upon birth, the digestive tracts of all animals are naturally

colonized by a variety of microorganisms (Savage, 1987). Under healthy and non-stressful conditions, "beneficial" microflora colonizes the rumen and lower gut in a symbiotic relationship with the host. Beneficial rumen and gut microorganisms supply nutrients to the host, aid in digestion of dietary nutrients and compete with potential pathogens. In contrast, when young animals are removed and raised under sterile conditions, microorganisms from the environment are prevented from colonizing their digestive tracts. These animals often have increased nutritional needs (e.g., requiring more vitamin K in the diet) and abnormal immune responses. Sterile animals also are more susceptible to bacterial infections, presumably due to rapid establishment of pathogens.

The original concept of administering direct fed microbial to animals was to feed large amounts of "beneficial" microbes to livestock when they were "stressed." This practice would prevent the establishment of pathogenic microorganisms and could help re-establish normal gut microflora. This practice was termed "probiotic" or "for life." The term "probiotic" implied a curative nature of these products that would require government approval in order to make legal product claims (e.g., decreased mortality, fewer sick days, and increased production).

One of the most common hypotheses that may explain how probiotics improve animal performance suggests that the addition of beneficial bacteria exclude the establishment of pathogens (competitive exclusion). Production of antimicrobial end products such as acids and antibiotics are also commonly discussed.

Bacterial DFM

Probiotics are live micro organisms fed directly to the animals. Thus, they are also known as direct fed microbials (DFM). In general, most would agree that DFM based on bacteria must be "live." Thus, they must survive processing, storage and the gut environment. However, future research may prove that end products such as bacteriocins (narrow spectrum antimicrobial substances) and not the actual organism itself may be beneficial. A list of some common bacteria that have potential as DFM additives is shown in Table 3.1.

Lactobacillus acidophilus (and other Lactobacillus species), *L. casei, Enterococcus diacetylactis*, and *Bacillus subtilis* are commonly used as DFM products for ruminants. These organisms appear to have little effect on ruminal fermentation (Ware *et. al*., 1988) and the site of action from these organisms appears to be in the lower gut but solid and repeatable data is lacking. Initial research with these organisms in ruminants was first centered on "stressed" animals with the general assumption that feeding beneficial organisms would decrease or prevent intestinal establishment of pathogenic microorganisms (Vandevoorde *et al*., 1991). In addition, it was hypothesized that massive doses of beneficial organisms would re-colonize a "stressed" intestinal environment and return gut function to normal more quickly. In ruminants, much of this research involved feeding *lactobacillus*-based DFM to young calves fed milk, calves being weaned or shipped cattle (Jenny *et al*., 1991; Hutcheson *et al*., 1980) because these conditions were often classified as times of high stress. Calves fed *L. acidophilus* have been reported to have reduced incidence of diarrhea (Beecham *et al*., 1977) and reduced counts of intestinal coliform bacteria (Bruce *et al*., 1979). Data summarizing more than 30 trials with incoming feedlot cattle showed an advantage of 10.7 and 5.4% in average daily gain and feed efficiency, respectively, for cattle fed a DFM (Pioneer Hi-Bred International, 1988). Only a few studies have documented positive effects of feeding bacterial DFM to lactating dairy cows. High producing cows in early lactation would be the best candidates for such products because these cows are in negative energy balance and have diets that contain highly fermentable carbohydrates that sometimes lead to acidosis. Jaquette *et al*. (1988) and Ware *et al*. (1988) reported increased milk production from cows fed *L. acidophilus* (1 x 109 colony-forming units per head per day). It has also been reported improvements in milk production when cows were fed a DFM containing yeast and 2 strains of bacteria. Supplementation of lactobacilli may be useful in the close-up dry period of lactation when intake is depressed and animals are stressed. However, there is limited data to support this use.

To some extent, the practice of using DFM on farm is already being used on many dairies. Specifically, producers and veterinarians have been inoculating sick ruminants with rumen fluid from healthy animals in hopes of stimulating normal rumen function for improving dry matter intakes has been practiced for decades. Several attempts have been made to use bacteria to alter rumen metabolism but only a few have been successful on a practical scale.

The detoxification of the 3-hydroxy-4(1H)-pyridone (DHP) by *Synergistes jonesii*, isolated from Hawaiian cattle, is probably one of the most cited successes of manipulating ruminal fermentation with bacteria. The tropical forage *Leucaena leucocephala* (Subabul in Hindi) contains mimosine, a non-protein amino acid. When consumed by ruminants in Australia and some parts of India, DHP causes goitrogenic effects. Jones and Megarrity (1986) showed that rumen microbes, from cattle in Hawaii, were able to detoxify DHP. The specific organism responsible for detoxification, *S. jonesii* (Allison *et al*., 1990), was inoculated and established itself in the rumen of Australian cattle thus conferring protection from DHP toxicity. Another problem in feeding ruminants, identified in Australia, is monofluroacetate. This compound is found in some Australian plants and can be toxic to ruminants at doses of about 0.3 mg/kg of body weight. Gregg *et al*. (1998) reported that they successfully inserted the gene encoding for fluoroacetate dehalogenase into several strains of *Butyrivibrio fibrisolvens* and when sheep were inoculated with the altered microbes, they showed reduced toxicological symptoms. However, use of the genetically modified rumen bacteria in the field is not currently approved.

Megasphaera elsdenii (ME) is the major lactate-utilizing organism in the rumen of adapted cattle fed high grain diets. However,

when cattle are abruptly shifted from a high-forage to high-concentrate diet, the numbers of ME are often insufficient to prevent lactic acidosis. We have shown that during a challenge with highly fermentable carbohydrates, addition of *Megasphaera elsdenii* B159 prevented an accumulation of lactic acid and shifted ruminal fermentation away from acetate and propionate towards butyrate and valerate (Kung and Hession, 1995). Addition of ME has also experimentally prevented acidosis in steers (Robinson *et al*., 1992). Development of this organism for feedlot cattle, and perhaps high producing dairy cows, should be continued with emphasis on optimizing dose and timing of administration. Success with such an organism could allow feedlot producers to decrease the time it takes to adapt cattle to a high concentrate diet. It could also be useful by reducing chronic acidosis in lactating cows.

Some *Propionibacteria* are naturally found in high numbers in the rumen of animals fed forage and medium concentrate diets. These organisms convert lactate and glucose to acetate and propionate. *Propionibacteria* may be beneficial if inoculated into the rumen (Kung *et al*., 1991) because higher concentrations of ruminal propionate would be absorbed into the blood and converted to glucose by the liver of the host animal. Although *Propionibacteria* can metabolize lactic acid, they are probably too slow growing and acid intolerant to prevent a challenge that would lead to acidosis. A commercially available product based on a strain of *Propionibacteria* that naturally occurs in the rumen has been claimed to reduce the chance of nitrate toxicity but definitive data is lacking. Recently, Swinney-Flyod *et al*. (1999) reported that feedlot cattle fed a diet containing *Propionibacteria*, strain P-63 (1 × 109 cfu/head/day) and *L. acidophilus*, strain 5345, (1 × 108cfu/head/day) had better feed efficiencies during adaptation to a high concentrate diet and during a 120-d feeding period. Similarly, Huck *et al*. (1999) reported that cattle fed *L. acidophilus* (5 × 108 cfu/head/day) strain BG2F04, and *P. freudenrechii* (1 × 109 cfu/head/day) had better feed efficiencies than those fed a control diet. More research in these areas is warranted.

Fungal DFM

A variety of mechanisms have been put forth to explain changes in ruminal fermentations and improvements in performance when ruminants are fed fungal-based DFM. For example, yeast may have a buffering effect in the rumen by mediating the sharp drops in rumen pH, which follows feeding. Martin and Streeter (1995) suggested that fungal cultures improve the use of lactate by the ruminal organism *Selenomonas ruminantium* by providing a source of dicarboxcylic acids (e.g., malic acid) and other growth factors. Thus, yeast may help to buffer excess lactic acid production when ruminants are fed high concentrate diets. The effects on buffering are subtle; as added yeast cannot prevent lactic acidosis if the rumen is challenged with a diet rich in fermentable carbohydrates (Aslan *et al*., 1995; Dawson and Hopkins, 1991). However, a higher pH may be one reason for the finding of increased numbers of rumen cellulolytic bacteria and improvements in fiber digestion with fungal cultures (Arambel *et al*., 1987). Newbold *et al*. (1995b) reported that the stimulation of rumen bacteria by *Saccharomyces cerevisiae* different with specific strains. Some fungal extracts have been suggested to contain esterase enzymes that may improve fiber digestion (Varel *et al*., 1993). Yeast may also stimulate rumen fermentation by scavenging excess oxygen from the rumen (Newbold *et al*., 1996). They have also been shown to stimulate acetogenic bacteria in the presence of methanogens (Chaucheryas *et al*., 1995). The effect of fungal cultures on ruminal VFA has been inconsistent. Newbold (1995a) summarized the literature and reported that fungal extracts had no effect or tended to increase the rumen acetate:propionate ratios while active yeast either had no effect or decreased the acetate:propionate ratio. Arizona researchers reported that feeding AO to cows in hot environments decreased rectal temperatures in some but not all studies (Huber *et al*., 1994). There is no direct evidence that yeast or fungal extracts affect digestion or metabolism in the lower gut. However, the potential for such effects have not been well studied.

The need for high numbers of live fungal organisms in fungal DFM additives has been the subject of many debates. Some products guarantee live yeast cells (e.g., 1 x 109 cfu per g) and are fed at low inclusion rates (only 10-20 grams per day) but other products suggest that live organisms are not required for beneficial effects because end products present in the additives are the “active” ingredients. Newbold *et al*. (1991) reported that autoclaving, but not irradiation, decreased the ability of an AO extract to stimulate rumen bacterial growth and activity. Dawson *et al*. (1990) reported that the stimulatory effect of yeast on numbers of rumen cellulolytic bacteria was negated when yeasts were autoclaved. Martin and Nibs (1992) reported that unpublished data from their lab showed enhanced uptake of D-lactate by *S. ruminantium*was enhanced by a filtrate from AO but not from SC. Although there have been implications that suggests yeasts were able to grow in continuous rumen cultures (Dawson *et al*., 1990) others have observed that live yeasts are essentially washed out of ruminal fermentations. We reported that *Saccharomyces cerevisiae* did not multiply in sterile ruminal fluid, but they did survive and were metabolically active (Kung *et al*., 1996).

In contrast to research with bacterial DFM, there is much data on the effect of feeding fungal DFM to lactating cows. In a review of 32 lactation comparisons conducted with yeast between 1986 and 1997, these supplements increased milk production on average by more than 1.13 kg (2.49 lb.) per day with the response being greater for cows in early lactation. Response appeared to be consistent over the years. In a summary of 26 comparisons where fungal extracts (from *Aspergillus oryzae*) were fed to lactating ruminants, we found an average increase in milk production of only 0.45 kg (1.01 lb.) of milk per day. Unexplainably, since 1991, milk production responses from fungal extracts (AO) have been relatively poor. Fungal cultures have also been fed to calves, sheep, and steers but applications with these species have been less researched than with lactating cows. For example, Beharka *et al*.

(1991) reported that young calves fed an AO fermentation extract were weaned one wk earlier than untreated calves and that supplementation increased the numbers of rumen bacteria and VFA concentrations.

From a practical point, fungal additives appear to be more useful when fed to cows in early lactation that are consuming high quantities of grain,

Practical Considerations for DFM/Probiotics

Direct-fed microbial products are available in a variety of forms including powders, pastes, boluses, and capsules. In some applications, DFM may be mixed with feed or administered in the drinking water. However, use of DFM in the latter manner must be managed closely since interactions with chlorine, water temperature, minerals, flow rate, and antibiotics can affect the viability of many organisms. Non-hydroscopic whey is often used as a carrier for bacterial DFM and is a good medium to initiate growth. Bacterial DFM pastes are formulated with vegetable oil and inert gelling ingredients. Some fungal products are formulated with grain by-products as carriers. Some DFM are designed for one-time dosing while other products are designed for feeding on a daily basis. However, there is little information comparing the efficacy of administering a DFM in a single massive dose compared to continuous daily dosing. Lee and Botts (1988) reported that pulse dosing alone or pulse dosing with daily feeding of *Streptococcus faecium* M74 resulted in improved performance of incoming feedlot cattle. The need for a bacterial DFM to actually attach and colonize gut surfaces in order to have a beneficial effect is also questionable. However, in certain applications, the argument could be made that a DFM organism need only produce its active component (without colonization) to be beneficial. Dose levels of bacterial DFM have varied. Studies can be found where *L. acidophilus* has been fed at levels ranging from 106 to 1010 cfu per animal per day. Hutchenson *et al*. (1980) suggested that feeding more than 107 cfu per head per day may cause lower nutrient absorption due to overpopulation of the gut. Orr *et al*. (1988) reported that feeding a continuous high dose of *L. acidophilus* to feeder calves (1010 cfu per head/day) had no effect on gain and actually reduced feed efficiency when compared to feeding a lower dose (106).

Tolerance of DFM microorganisms to heat is important since many feeds are pelleted. In general, most yeast, *Lactobacillus*, *Bifidobacterium*, and *Streptococcus* are destroyed by heat during pelleting. In contrast, bacilli form stable endospores when conditions for growth are unfavorable and are very resistant to heat, pH, moisture and disinfectants. Thus, bacilli are currently used in many applications that require pelleting. Over-blending can sometimes compensate for microbial loss during pelleting, but this is not an acceptable routine practice. Future improvements in strain development may allow use of heat sensitive organisms in pelleted feeds. Bacterial products may or may not be compatible with use of traditional antibiotics and thus care should be taken when formulations contain both types of additives. Information on DFM and antibiotic compatibility should be available from the manufacturer. For example, some species of bacilli are sensitive to virginiamycin, and lactobacilli are sensitive to chlortetracycline and penicillin. Viability of DFM products has improved over the past several years but it is highly advisable to adhere to storage recommendations. For example, products should be kept away from moisture, excess heat, and light.

Table 3.1. Some bacteria that have potential uses as Probiotics

Sr. No.	Organisms	End Products or Potential Use
1.	*Lactobacillus acidophilus*	Lactic acid, acidophilin, glycosidases
2.	*Pediococsus acidilactici*	Pediocin (bacteriocin)
3.	*L. lactis*	Amylase, hydrogen peroxide, proteases
4.	*Bifidobacterium bifidum*	Ureases, lactic acid, formic acid
5.	*Bacillus subtilis*	Amylase, protease
6.	*Propionibacterium thoenii*	Propionicin PLG-1 (bacteriocin)
7.	*Megasphaera elsdenii*	Ruminal lactate utilizer
8.	*Propionibacteria sp.*	Ruminal lactate utilizer, propionate producer

Conclusion

Feeding of probiotics may have impact on the overall performance of the livestock and poultry. However, the results may not be well pronounced always especially if the diets are adequate in all the nutrients and also the animals are not in stress condition. Responses to a growth promoter depend on a variety of factors *viz*. product composition, malnutrition, stress condition, health of

animals, challenge from variant strains of pathogens etc. When these factors exist, probably the beneficial effects of probiotics could be significant. Further, studies are needed to evaluate the probiotics with different combinations in different agroclimatic conditions. In addition, detail studies should be carried out to develop standard packages comprising of ideal combinations of probiotics and prebiotics (synbiotics) for obtaining optimum performance of livestock and poultry under different agro climatic conditions.

References

Allison, M. J., Hammond, A.C. and Jones, R.J. 1990. Detection of rumen bacteria that degrade toxic dihydroxypridine compounds produced from mimosine. *Applied and Experimental Microbiology*, **56**: 590-594.

Arambel, M. J., Weidmeier, R.D. and Walters, J. L. 1987. Influence of donor animal adaptation to added yeast culture and/or *Aspergillus oryzae* fermentation extract on in vitro rumen fermentation. *Nutrition Reports International*, **35**: 433-437.

Aslan, V. S., Thamsborg, M., Jorgensen, R. J. and Basse, A. 1995. Induced acute ruminal acidosis in goats treated with yeast (*Saccharomyces cerevisiae*) and bicarbonate. *Acta Veterinaria Scandinavica*, **36**: 65-68.

Banday, M.T. and Risam, V.S. 2001. Growth performance and carcass characteristics of broiler chicken fed with probiotics. *Indian Journal of Poultry Science*, **36**: 252-255.

Barrow, P.A. 1992. Probiotic for chickens. In Fuller R. (Ed), Probiotics. The Scientific basis. Chapman and Hall, London, pp: 225-257.

Beecham, T. J., Chambers, J. V. and Cunningham, M.D. 1977. Influence of *Lactobacillus acidophilus* on performance of young dairy calves. *Journal of Dairy Science*, **60**: 74. (Abstract)

Beharka, A. A., Nagaraja, T. G. and Morrill, J. L. 1991. Performance and ruminal development of young calves fed diets with *Aspergillus oryzae* fermentation extracts. *Journal of Dairy Science*, **74**: 4326-4336.

Bruce, B. B., Gilliland, S.E., Bush, L. J. and Staley, T. E. 1979. Influence of feeding cells of *Lactobacillus acidophilus* on the fecal flora of young dairy calves. *Oklahoma Animal Science Research Reports*, 207.

Chaucheryras, F., Fonty, G., Bertin, G. and Gouet, P. 1995. *In vitro* utilization by a ruminal acetogenic bacterium cultivated alone or in association with an Archea methanogen is stimulated by a probiotic strain of *Saccharomyces cerevisiae*. *Applied and Environmental Microbiology*, **61**: 3466-3467.

Dalloul, R.A., Lillehoj, H.S., Shellem, T.A. and Doerr, J.A. 2003. Intestinal immunomodulation by vitamin A deficiency and lactobacillus-based probiotic in Eimeria acervulina-infected broiler chickens. *Avian Diseases*, **47**: 1313-1320.

Dawson, K. A., and Hopkins, D. M. 1991. Differential effects of live yeast on the cellulolytic activities of anaerobic ruminal bacteria. *Journal of Animal Science*, **69**: 531. (Abstract).

Dawson, K. A., Neuman, K. E. and Boling, J. A. 1990. Effects of microbial supplements containing yeast and lactobacilli on roughage-fed ruminal microbial activities. *Journal of Animal Science*, **68**: 3392-3398.

Fuller, R. 1995. Probiotic strains and health. *I.D.F. Nut. News letter*, **145**:29.

Gregg, K., Hamdorf, B., Henderson, K., Kopecny, J. and Wong, C. 1998. Genetically modified ruminal bacteria protect sheep from fluoroacetate poisoning. *Applied and Environmental Microbiology*, **64**: 3496-3498.

Huber, J. T., Higginbotham, G., Gomez-Alarcon, R. A., Taylor, R. B., Chen, K. H., Chan, S.C. and Wu, Z.. 1994. Heat stress interactions with protein, supplemental fat and fungal cultures. *Journal of Dairy Science*, **77**: 2080-2090.

Huck, G. L., Kriekemeier, K. K. and Ducharme, G. A. 1999. Effect of feeding *Lactobacillus acidophilus* BG2F04 (Micro cell) and *Propionibacterium freudenrechii* P -63 (MicroCell PB on growth performance of finishing heifers. *Journal of Animal Science*, **77**: 264.

Hutchenson, D.P., Cole, N.A., Keaton, W., Graham, G., Dunlap, R. and Pitman, K. 1980. The use of living, nonfreeze-dried *Lactobacillus acidophilus* culture for receiving feedlot calves. *Proceedings, Western Section, American Society of Animal Science*, **31**: 213.

Huthail, N. and Najib. H. 1996. Effect of incorporating yeast culture *Saccharomyces cerevisiae* into Saudi Baladi and White Leghorn Layers diet. *Journal of Applied Animal Research*, **10**: 181-186.

Jaquette, R. D., Dennis, R. J., Coalson, J. A., Ware, D. R., Manfredi, E. T. and Read, P.L. 1988. Effect of feeding viable *Lactobacillus acidophilus* (BT1386) on the performance of lactating dairy cows. *Journal of Dairy Science*, **71**: 219.

Jenny, B. F., Vandijk, H. J. and Collins, J. A. 1991. Performance and fecal flora of calves fed a *Bacillus subtilis* concentrate. *Journal of Dairy Science*, **74**: 1968-1973.

Jin, L.Z., Ho, Y.W., Abdullah, N. and Jalaludin, S. 1997. Probiotic in poultry: Mode of action. *World's Poultry Science Journal*, **53**: 351-368.

Jones, R. J., and Megarrity, R.G. 1986. Successful transfer of DHP-degrading bacteria from Hawaiian goats to Australian ruminants to overcome the toxicity of *Leucaena*. *Australian Veterinary Journal*, **63**: 259-262.

Kahraman, R. Alp, M., Kocabogli, N., Abas, I., Aksu, H. and Taner, A. 1997. Effects of probiotic supplementation to the oxidized diets on performance, ileal pH and Enterobacteriaceae population, ascites incidence and mortality rate of broilers. *Pendik*

Veteriner Mikrobiyoloji Dergisi, **28**: 181-190.
Krueger, W. F., Kassogue, A. and Fanguy, R.C. 1990. Effect of yeast added to the diet of broilers on performance to 28 and 49 days of age. *Poultry Science*, **69**: 75 (Abstract).
Kung, L., Jr. and Hession, A. O. 1995. Altering rumen fermentation by microbial inoculation with lactate-utilizing microorganisms. *Journal of Animal Science*, **73**: 250-256.
Kung, L., Jr., Hession, A., Tung, R.S. and Maciorowski, K. 1991. Effect of *Propionibacterium shermanii* on ruminal fermentations. *Proceedings of 21st Biennial Conference on Rumen Function*. Chicago, IL, p 31. (Abstract)
Kung, L., Jr., Kreck, E.M., Tung, R.S., Hession, A. O., Sheperd, A. C., Cohen, M. A., Swain, H. E. and Leedle, J.A.Z. 1996. Effects of a live yeast culture and enzymes on *in vitro* ruminal fermentation and milk production of dairy cows. *Journal of Dairy Science*, **80**: 2045-2051.
Kurtoglu,V., Kurtoglu. F., Seker, E., Coskun, B., Balevi, T. and Polat, E.S. 2004. Effect of probiotic supplementation on laying hen diets on yield performance and serum and egg yolk cholesterol. *Food Additives and Contaminants*, **21**: 817-823.
Lee, R. W. and Botts, R.L. 1988. Evaluation of a single oral dosing and continuous feeding of *Streptococcus faecium* M74 (Syntabac) on the performance of incoming feedlot cattle. *Journal of Animal Science*, **66**: 460. (Abstract)
Marriott, W., Mckim and Davidson, J. T. 1923. Acidified whole milk as a routine infant food. *Journal of American Medical Association*, **81**: 2007- 2009.
Martin, S. A. and Nisbet, D. J. 1992. Effect of direct-fed microbials on rumen microbial fermentation. *Journal of Dairy Science*, **75**: 1736-1744.
Martin, S. A. and Streeter, M. N. 1995. Effect of malate on *in vitro* mixed ruminal microorganism fermentation. *Journal of Animal Science*, **73**: 2141-2145.
Newbold, C. J. 1995a. Microbial feed additives for ruminants. In: Biotechnology in Animal Feeds and Animal Feeding. R. J. Wallace and A. Chesson (Eds.). VCH. New York. Pp. 259-278.
Newbold, C. J., Brock, R. and Wallace, R. J. 1991. Influence of autoclaved or irradiated *Aspergillus oryzae* fermentation extract on fermentation in the rumen simulation technique (Rusitec). *Journal of Agricultural Science*, **116**: 159-162.
Newbold, C. J., Wallace, R. J. and McIntosh, F.M. 1996. Mode of action of the yeast *Saccharomyces cerevisiae* as a feed additive for ruminants. *British Journal of Nutrition*, **76:** 249.
Newbold, C. J., Wallace, R. J., Chen, X. B. and McIntosh, F. 1995b. Different strains of *Saccharomyces cerevisiae* differ in their effects on ruminal bacterial numbers *in vitro* and in sheep. *Journal of Animal Science*, **73**:1811-1818.
Panda, A.K., Rao, S.S.R., Raju, M.V.L.N. and Sharma, S.S. 2008. Effect of probiotic (*Lactobacillus sporogenes*) feeding on egg productuion and quality, yolk cholesterol and humoral immune response of White Leghorn layer breeders. *Journal of Science of Food and Agriculture*, **88:** 43-47.
Panda, A.K., Reddy, M.R. and Praharaj, N.K. 2001. Dietary supplementation of probiotic on growth, serum cholesterol and gut microflora of broilers. *Indian Journal of Animal Science*, **71**: 488-490.
Pioneer Hi-bred International. 1988. Summary of the overall effect of Probios brand microbial products on the performance and health of incoming feedlot cattle. *Pioneer Hi-bred International*, Johnston, IA.
Robinson, J. A., Smolenski, W.J., Greening, R.C., Ogilvie, R. L., Barsuhn, B.K. and Peters, J.P. 1992. Prevention of acute acidosis and enhancement of feed intake in the bovine by *Megasphaera elsdenii*407A. *Journal of Animal Science*, **70**: 310 (Abstract).
Savage, D. C. 1987. Microorganisms associated with epithelial surfaces and the stability of the indigenous gastrointestinal microflora. *Die Nahrung*, **5-6**: 383.
Sharma, K.S., Sharma, K.V., Kumar, M., Shavani, K., Mukul, K.S. and Katoch, B.S. 2001. Performance of laying pullets fed microbial combinations upto eighteen weeks of age/ the age of sexual maturity. *Indian Journal of Animal Science*, **71**: 566-569.
Sperti, G. S. 1971. Probiotics. AVI Publishing Co. Inc., West Point, Connecticut.
Swinney-Floyd, D, Gardner, B.A., Rehberger, T. and Parrot, T. 1999. Effects of inoculation with either *Propionibacterium* strain P-63 alone or comb0ined with *Lactobacillus acidophilus* strain: LZ 53545 on performance of feedlot cattle. *Journal of Animal Science*, **77:** 77 (Abstract).
Tibiletti, E. 1993. Combatting stress with probiotics. *Rivista di Avicoltura*, **62**: 19-21.
Vandevoorde, L., Christianens, H. and Verstraete, W. 1991. *In vitro* appraisal of the probiotic value of intestinal lactobacilli. *World Journal of Microbiology In addition, Biotechnology*, **7**: 587-592.
Varel,V.H., Kreikemeier, K. K., Jung, H.F.G. and Hatfield, R. D. 1993. *In vitro* stimulation of forage fiber degradation by ruminal microorganisms with *Aspergillus oryzae* fermentation extract. *Applied and Environmental Microbiology*, **59**:3171-3176.
Ware, D. R., Read, P. L. and Manfredi, E. T. 1988. Lactation performance of two large dairy herds fed *Lactobacillus acidophilus* strain BT 1386. *Journal of Dairy Science*, **71**: 219 (Abstract).
Yakout, H.M., Omara, M. E. Marie, Y. and Hasan, R.A. 2004. Effect of incorporating growth promoters and different dietary

protein levels into Mandarah hens layer's diets. *Egyptian Poultry Science Journal*, **24**: 977-994.
Yalcin, S., Yalcin S. and Kocak, D. (1993). Effects of dietary baker's yeast on meat quality of broiler chicks. *Veteriner Fakultesi Dergisi* **40**: 577-585.

4 Prebiotics

Prebiotic foods have been consumed for centuries, either as natural components of food, or as fermented food. However, interest in dietary use of prebiotics blossomed in the later 1800s and early 1900. Preliminary researches revealed that lactose had a profound effect on the pH of the caecal contents and intestine due to lactic acid fermentation resulting in enhanced calcium and phosphorous absorption. Further, it was also noted by several workers that inclusion of lactose in poultry diets resulted in better growth and reduced mortality.

A prebiotic can be defined as "a non digestible food ingredient that beneficially affects the host by selectivity stimulating the growth and/or activity of one or limited number of bacteria in the colon, and thus improves host health" (Gibson and Roberfroid, 1995). Based on this definition, Russell (1998) formulated the criteria according to which a substance can be a prebiotic. First, prebiotics are always feed ingredients that are not digested by the host, not or little used and/or metabolised as they pass through the upper portion of the intestinal tract, so they can reach the flora of the large intestine. Secondly, they have to be able to serve as a substrate for one or more bacterial species with a potentially beneficial effect on the host. Finally they have to be able to cause a shift in the microflora that improves the health of the host. In principle only non-digestible, fermentable feed components are prebiotics.

Table 4.1. Intestinal functions assigned to prebiotics

Dietary fibers and gastrointestinal functions	
Effects on upper GI tract	• Resistance to digestion
	• Retarded gastric emptying
	• Increased oro-caecal transit time
	• Reduced glucose absorption and low glycaemic index
	• Hyperplasia of the small intestinal epithelium
	• Stimulation of secretion of intestinal hormonal peptides
Effects on lower GI tract	• Acting as food for colonic microbiota
	• Acting as substrates for colonic fermentation
	• Production of fermentation end products (mainly SCFAs)
	• Stimulation of saccharolytic fermentation
	• Acidification of the colonic content
	• Hyperplasia of the colonic epithelium
	• Stimulation of secretion of colonic hormonal peptides

- Bulking effect on stool production
- Regularization of stool production (frequency and consistence)
- Acceleration of ceco-anal transit

A combination of probiotics and prebiotics is called a synbiotic (Collins and Gibson, 1999; Schrezenmeir and de Vrese, 2001). This combination could improve the survival of the probiotic organism, because its specific substrate is available for fermentation. Examples of synbiotics are FOS and *bifido bacteria*, and lactitol and *lactobacilli*.

Characteristics of Ideal Prebiotics

1. It should be neither hydrolysed nor absorbed by mammalian enzyme or tissues.
2. It must selectively enrich for one or a limited number of beneficial bacteria.
3. It should beneficially alter the intestinal microbiota and their activities.
4. It should beneficially alter luminal or systemic aspects of the host defense system.

Beneficial Effects of Prebiotics

There are several beneficial effects of prebiotics. They are as follows:

1. It increases biomass and stool bulking
2. It enhances vitamin-B synthesis.
3. It improves mineral absorption.
4. It increases production of VFA.
5. It prevents cancer
6. It lowers serum cholesterol

Besides these beneficial effects, prebiotics have several others, which have been illustrated in recent times. Prebiotics have been found to minimize the incidence of ascites (Santos *et al*., 2005). Prebiotics have been also found to lower egg cholesterol (Chen *et al*., 2005a), increase egg production and feed efficiency of layers (Chen *et al*., 2005b) and improve eggshell quality (Chen and Chen, 2004).

Classification

The term prebiotics is generally restricted to indigestible carbohydrates. These carbohydrates are divided in groups based on their molecular length: mono di, oligo and polysaccharides.

The most important monosaccharide prebiotics are hexoses (glucose, fructose, galactose, mannose) and pentoses (ribose, xylose, arabinose). Monosaccharides such as glucose and fructose are digestible as monomers and therefore not prebiotics according to the definition of Russell (1998). Galactose is available mostly under the disaccharide feed additive. These monosaccharides can form the basis for enzymatically constructed oligo or polysaccharides.

The most important natural disaccharides are sucrose, lactose and maltose. Isomerization products of these compounds can be used as prebiotics e.g. lactulose (based on lactose). Lactose, lactulose and lactosucrose have prebiotic effects in chickens.

The most important polysaccharide prebiotic for chickens is guar gum, produced from the seeds of the guar bean, *Cyamopsis tetragonolobus*. Another possible classification is based on their source of origin: natural or synthetic saccharides (Iji and Tivey, 1998). Examples of natural sources of oligosaccharides are soybeans, oil palm kernels, white lupin, blue lupin, chick pea and black gram. The different classes of synthetic oligosaccharides are Lactulose, Mannan-oligosaccharides and Isomalto-oligosaccharides etc. The list of different types of oligosaccharides is quite long. However only those that have been used in poultry have been discussed in this paper.

Mechanism of Action of Prebiotics

Prebiotics can have a direct effect either by direct binding of pathogens or by increasing the osmotic value in the intestinal lumen. More often, however, their effects are indirect, mediated by metabolites, which are generated by the intestinal flora that uses the

prebiotics for their own metabolism. Such metabolites include short-chain fatty acids, lactate, polyamines and bactericins.

Type 1 (F1) fimbria has been described in several bacterial enteric pathogens. These type I fimbriae bind to mannose residues of glycoproteins, present on the surface of eukaryotic cells. They are needed for the adhesion of the bacteria to the mucosal surface, which is a prerequisite for the colonization of the host. Indigestible carbohydrates with mannose residues may bind the F1 fimbriae and therefore block the adhesion of the bacteria to the epithelial cells (Finucane *et al*., 1999). Prebiotics may constitute a substrate for the growth of intestinal flora. This multiplication of normal flora may inhibit the colonization with pathogenic bacteria. This phenomenon of inhibition is called "competitive exclusion". Another possible mechanism of action of prebiotics is through a modification of the metabolic activity of normal intestinal flora. Saccharides, indigestible by the host, are fermented by the flora into volatile fatty acids (VFA including acetate, propionate and butyrate), lactate and several gases including carbondioxide, methane and hydrogen. These acids lower the pH of the medium and may thus exert antibacterial effect. An added effect of acid production is the protonation of potentially toxic ammonia (and amines) to produce NH4+, which is nondiffusable and thus lowers blood ammonia levels.

Ruminants

The use of prebiotics in cattle has been limited due to the ability of ruminants to degrade most prebiotics; however enhancements in rumen-protective technologies may allow these compounds to be used in feedlot and dairy cattle (Callaway *et al*., 2008), considering also that several classes of nondigestible oligosaccharides are found in plant cell wall in nature including feeds normally used for livestock feeding (Lema *et al*., 2002). Addition of MOS to the diet of Holstein calves improved fecal scores just as for antibiotic treatment when compared to control milk replacer; whereas body weight was not affected (Heinrichs *et al*., 2003). Supplementation of sorbitol, L-arabinose, trehalose, and rhamnose to cattle rumen medium displaced E. coli O157:H7 within 72 h (De Vaux *et al*., 2002). The overall studies on the effect of forage and concentrate diets on fecal shedding and colonization of the gut by E. coli O157:H17 are still unclear and little information is available; however the manipulation of the fiber content could bring new perspectives maintaining the animals on a concentrate diet without sacrificing cattle weight gain as showed by Lema *et al*. (2002).

Pigs

TOS included at 35 g/kg in a diet for growing pigs resulted in a significant increase in fecal bifidobacteria and lactobacilli without growth performance increase (Smiricky-Tjardes *et al*., 2003). A novel galactooligosaccharide (GOS) mixture, supplied at 40 g/kg diet, resulted in a significant increase of the density of bifidobacteria and acetate concentration, and in a decrease of pH compared with the control diet and a control diet supplemented with inulin. In addition, the oligosaccharide mixture strongly inhibited the attachment of ETEC E. coli and S. enterica serotype typhimurium to HT29 cells *in vitro* (Tzortzis *et al*., 2005). An interesting study was conducted on the effects of barley and oat cultivars, with different carbohydrate compositions, on the intestinal bacterial communities in weaned piglets. Increased levels of â-glucans and altered amylopectin/amylose ratio seemed to selectively promote butyrate-producing bacteria, able to hydrolyzed complex carbohydrates. Furthermore, bifidobacteria and lactobacilli counts were positively affected by the choice of the cereal variety (Pieper *et al*., 2008). Oligosaccharides incorporated into swine diets at levels ranging from 5 to 40 g/kg diet have resulted in mixed but generally not significant effects regarding beneficial modulation of microbial populations determined in various intestinal segments and feces of swine (Mikkelsen *et al*., 2003). Mountzouris *et al*. (2006) showed that the dietary treatment with fructooligosaccharides (FOS) or trans-galactooligosaccharides (TOS) did not influence the populations of the beneficial bacterial but promoted saccharolytic activities in the porcine colon basing on the value of total volatile fatty acids, acetate concentrations and glycolytic activities. Modesto *et al*. (2009) reported that GOS from milk whey, and sugar beet fructooligosaccharides (sbFOS) added to the diet of weaned pigs in different amounts had no effect on the hindgut microbiota, except for SbFOS at 40 g/kg which tended to increase the endogenous bifidobacteria, whereas growth performance was not influenced.

Poultry

Lactose

Lactose is generally of animal origin and is found in the milk of mammals. Human milk contains 6% lactose and cows' milk 4.5%. Lactose is not hydrolyzed or absorbed intact from the intestinal tract of chicks, and as much as 50% of ingested lactose in poultry diets may be excreted unchanged. Because of its lack of digestion and absorption, lactose passes into the lower segments of the intestine and caeca. Lactose and its isomerization products such as lactulose reportedly have prebiotic effects in chickens.

Including lactose in the diet of chicken may produce significant improvement in body weight gain. It has been seen that lactose has a hypocholesteromic effect in quails (Gujral, 2005). This may be due to enhanced activity of lactic acid bacteria requiring cholesterol for their own metabolism resulting in decreased absorption of cholesterol in the gastro intestinal tract.

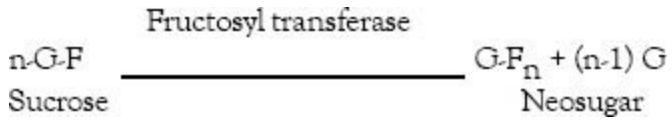
Mannan-oligosaccharide

Saccharomyces yeast outer cell wall components known as mannan-oligosaccharides (MOS) consists of Mannose and glucose polymer. Commercially it is available as Bio-MosTM manufactured by Alltech Inc., USA. The product contains yeast cell wall fragments derived from *Saccharomyces cerevisiae*. The cell wall fragments are obtained by centrifugation from a lysed yeast cell culture. The pellet containing the yeast cell wall fragments is then washed and spray dried.

There are several reports available on studies with MOS in poultry. It has been suggested that MOS may be an alternative to antibiotics in improving feed conversion (Fritts and Waldroup, 2000; Hulet *et al*., 2000). Supplementation of MOS may reduce abdominal fat and increase breast yield in broilers. Further, MOS may reduce the ammonia concentration in the cecal digesta of turkeys (Zdunczyk *et al*., 2005). It has been seen that MOS has immunomodulatory effect in birds. Inclusion of MOS @ 0.5g/ kg significantly enhanced serum antibody titre against sheep red blood cells and increased macrophage activity in chickens (Cotter *et al*., 2000; Shao *et al*., 2000). Savage and Zakrzewska (1997) reported increased concentration of bile IgA and plasma IgG in response to mannanoligosaccharides in turkey poults. Similarly including MOS in the diets of broilers containing aflatoxins have led to significant improvement in titre values against Newcastle disease and Infectious Bursal Disease (Swamy and Devegowda, 1998). MOS has also been found to lower the egg total cholesterol, serum total cholesterol and LDL cholesterol in layers when provided with 1.0 g/kg in the ration (Stanley and Sefton, 1999).

Fructo oligosaccharides

Fructo oligosaccharides (FOS) consist of a linear chain of B-D fructofuranose units linked 1, 2 by glycosidic bonds. Chains may terminate with a D glycopyranose unit at the non-reducing end. They can be extracted from plants like onions and asparagus, by controlled (limited) enzymatic hydrolysis of insulin polymers from chicory roots (Heinz and Vogel, 1991) and by enzymatic synthesis from sucrose using a fructosyl transferase from *Aspergillus niger* or *Aureobasidium pullalans* (Hidaka *et al*., 1988; Hidaka and Hirayama, 1991) according to the reaction:



Neosugar is a mixture of glucose, sucrose and FOS with a terminal glucose unit. Glucose and sucrose can be removed from the reaction mixture by chromatography to obtain a product with increased FOS purity.

FOS has elicited a great interest to improve intestinal health and productivity in poultry. FOS is known for their ability to stimulate growth of Bifidobacteria and to inhibit that of potentially pathogenic bacteria such as *Enterobacteria*, *Clostridia* and *Salmonella*. FOS has shown to be resistant to intestinal glycolytic enzymes and to pass unaltered to the large intestine where they are fermented by the microflora.

Significant improvement in weight gain, feed efficiency, mortality, carcass fat contents and dressing percentage have been observed with addition of FOS in broiler diets. Further, feeding FOS to broilers may reduce intestinal colonization by pathogens in birds.

It has been seen that FOS enhances the growth of intestinal bacterial organisms especially Lactobacillus species and Bifidobacterium. Some cellular components of Bifidobacteria promote immunological attack against malignant cells and thereby act as immunomodulators. Further, FOS also aids in amelioration of antibiotic associated diarrhoea and reduction of serum triglycerides and cholesterol. Growing female quails fed diet supplemented with FOS had higher lysozyme levels in blood serum (Szczerbinska *et al*., 2000) and egg white with no reduction in serum protein content (Tarasewicz, 1998). Further, female quails supplemented with FOS showed reduced contents of blood lipids and cholesterol (Tarasewicz, 1998). This may be due to the fact that acetate and propionate in combination with L-lactate play a role in regulating lipid and cholesterol metabolism.

Miscellaneous prebiotics

Partially Hydrolysed Guargum (PHGG)

The most commonly used polysaccharide prebiotic for chickens is guargum, produced from the seeds of the guarbean, *Cyamopsis tetragonolobus*. By selectivity cleaving the mannan back-bone chain of guar gum using endo-B-D-mannanase, a mixture of galactomannans is obtained, called PHGG. Preliminary studies reveal that PHHG may reduce microbial load in birds.

Lactosucrose

Broiler chicks on a diet supplemented with lactosucrose at 1.5 g/kg have been shown to have a reduced incidence of lecithinase-

negative bacteria and lower concentrations of ammonia than those on the control diet (Terada *et al*., 1994). Further, lactosucrose also increased the concentration of acetic and butyric acids in the ceca.

Isomalto oligosaccharide

Leuconostoc mesenteroides isomaltoolig saccharides (IMO) stimulated growth of *Bifido bacterium* and *Lactobacillus* and are not used by *Salmonella* or *E. coli* cecal isolates. Further, *Salmonella typhimurium* grown in mixed cultures on IMO reduced the *Salmonella* population. Cecal isolates grown on IMO showed higher viable counts and faster growth than *Salmonella*, indicating a potential value for these oligomers for poultry intestinal micro flora modification (Chung and Day, 2004).

Conclusion

A wide variety of prebiotics is commercially available as feed additives. When added in limited amounts to poultry feeds, they can result in a significant improvement in weight gain, efficiency of feed conversion and health status of the poultry. Further, it has been seen that several herb polysaccharides may serve as alternative for antimicrobial growth promoters in chicken (Guo, 2003). Turkeys are good forage feeders (Bhattacharyya *et al*., 2006). They are also resistant to different diseases unlike chicken in extensive or semi-intensive management system. Hence, extracts of these polysaccharides may serve as a growth promoter in different species of poultry. Prebiotics don't have a major role to play when optimum conditions of management and housing are provided. However, in practical conditions involving large flocks where there is deviation from the optimum conditions, prebiotics play a pivotal role in cost involved and economic return.

Several key advantages of these prebiotics are as follows:

1. Most are natural products, made of very simple sugars, without any antigenic capacity.
2. They can promote directly the growth of beneficial bacteria already present in the intestine
3. They have no viability constraints like micro-organisms (probiotics).
4. They do not present side effects or accumulate in tissues like antibiotics.
5. They are resistant to high temperatures and to the acidic pH of the proventriculus, which avoids formulation and application problems.
6. Their production cost is low and compatible with dose effect in poultry feed.

Elucidation of mechanism of action of each prebiotic will allow more accurate structure/function relationships to be established leading to the design of specific carbohydrate structures with improved efficacy.

References

Bhattacharyya, A., Majumdar, S., Bhanja, S.K. and Singh, R.P. 2006. Effect of feeding green berseem on growth and carcass characteristics of growing turkey poults. *Indian Journal of Poultry Science*, **41**: 58-63.

Callaway, T.R., Edrington, T.S., Anderson, R.C., Harvey, R.B., Genovese, K.J., Kennedy, C.N., Venn, D.W and Nisbet, D.J. 2008. Probiotics, prebiotics and competitive exclusion for prophylaxis against bacterial disease. *Animal Health Research Reviews*, **9:** 217–225.

Chen, Y.C. and Chen, T.C. 2004. Mineral utilization in layers as influenced by dietary oligofructose and inulin. *International Journal of Poultry Science*, **3:** 442–445.

Chen, Y.C., Nakthong, C. and Chen T.C. 2005a. Improvement of chicory fructans on egg cholesterol in commercial laying hen. *International Journal of Poultry Science*, **4:** 109–114.

Chen, Y.C., Nakthong, C. and Chen T.C. 2005b. Improvement of laying hen performance by dietary prebiotic chicory oligofructose and inulin. *International Journal of Poultry Science*, 4: 103-108.

Chung, C.H. and Day, D.F. 2004.Efficiency of *Leuconostoc mesenteroides* (ATCC 13146) isomalto oligosaccharides as poultry prebiotic. *Poultry Science*, **83**: 1302–1306.

Collins, M.D. and Gibson, R. 1999. Probiotics, prebiotics and synbiotics: approaches for modulating the microbial ecology of the gut. *American Journal of Clinical Nutrition*, **69**: 1052s–1057s.

Cotter,P.F., Malzone,A., Paluch, B., Lilburn, M.S.and Sefton, A.E. 2000. Modulation of humoral immunity in commercial laying hens by a dietary probiotic. *Poultry Science*, **79** (Suppl.1): 38 (Abstr.).

De Vaux, A., Morrison, M. and Hutkins, R.W. 2002. Displacement of Escherichia coli O157: H7 from rumen medium containing prebiotic sugars. *Applied and Environmental Microbiology*, **68**: 519–524.

Finucane, M., Spring, P. and Newman, K. 1999. Incidence of mannose sensitive adhesions in enteric bacteria. Abstracts 88th

Annual Meeting Poultry Science Association. 139.
Fritts, C.A. and Waldroup, P.W. 2000. Utilization of Bio-Mos mannan oligosaccharide in turkey diets. *Poultry Science*, **79** (Suppl.1): 29. (Abstr.).
Gibson, G.R., and Roberfroid, M.B. 1995.Dietary modulation of the human colonic microbiota introducing the concept of prebiotics. *Journal of Nutrition*, **125**:1401–1412.
Gujral, D. 2005. *Efficacy of certain oligosaccharides as substitute for dietary antibiotic for optimum performance of meat type quails*. Ph.D. Thesis, Deemed University, Indian Veterinary Research Institute, Izatnagar, U.P., India.
Guo, FuChun 2003. Mushroom and herb polysaccharides as alternative for antimicrobial growth promoters in poultry. *Mushroom and herb polysaccharides as alternative for antimicrobial growth promoters in poultry* 281.
Heinrichs, A.J., Jones, C.M. and Heinrichs, B.S. 2003. Effects of mannan oligosaccharide or antibiotics in neonatal diets on health and growth of dairy calves. *Journal of Dairy Science*, **86**: 4064–4069.
Hidaka, H. and Hirayama, M. 1991. Useful characteristics and commercial applications of fructo oligosaccharides. *Biochemical Society Transactions*, **19**: 561–565.
Hidaka, H., Hirayama, M. and Sumi, N. 1988. A fructo oligosaccharide producing enzyme from Aspergillus niger ATCC 20611. *Agricultural Biological chemistry*, **52**: 1181–1187.
Hulet, R.M., Loruz, E.S. and Saleh T.M. 2000. Turkey hen growth response to diets supplemented with either antibiotic manna oligosaccharide. *Poultry Science*, 79 (Suppl. 1): 124. (Abstr.).
Iji, P.A., and Tivey, D.R. 1998. Natural and synthetic oligosaccharides in broiler chicken diets. *World Poultry Science Journal*, 54: 129–143.
Lema, M., Williams, L., Walker, L. and Rao, D.R. 2002. Effect of dietary fiber on E. coli O157: H7 shedding in lambs. *Small Ruminant Research*, **43**: 249–255.
Mikkelsen, L.L., Jakobsen, M. and Jensen, B.B. 2003. Effects of dietary oligosaccharides on microbial diversity and fructo-oligosaccharide degrading bacteria in faeces of piglets post weaning. *Animal Feed Science and Technology*, **109**: 133–150.
Modesto, M., D'Aimmo, M.R., Stefanini, I., Trevisi, P., De Filippi, S., Casini, L., Mazzoni, M., Bosi, P. and Biavati, B. 2009. A novel strategy to select Bifidobacterium strains and prebiotics as natural growth promoters in newly weaned pigs. *Livestock Science*, **122**: 248–258.
Mountzouris, K.C., Balaskas, C., Fava, F., Tuohy, K.M., Gibson, G.R. and Fegeros, K. 2006. Profiling of composition and metabolic activities of the colonic microflora of growing pigs fed diets supplemented with prebiotic oligosaccharides. *Anaerobe*, **12**: 178–185.
Pieper, R., Jha, R., Rossnage, B., Van Kessel, A.G., Souffrant, W.B. and Leterme, P. 2008. Effect of barley and oat cultivars with different carbohydrate compositions on the intestinal bacterial communities in weaned piglets. *FEMS Microbiology Ecology*, **66**: 556–566.
Russell, Y.J. 1998. *The effect of natural source of non-digestible oligosaccharides on the fecal microflora of the dog and effects on digestion*. Friskies R&D Center, Missouri, U.S.A.
Santos, F.S. de los, Farnell, M.B., Tellez, G., Balog, J.M. and Anthony, N.B.2005. Effect of prebiotic on gut development and ascitis incidence of broilers reared in a hypoxic environment. *Poultry Science*, **84**: 1092–1100.
Savage, T.F. and Zakrezewska, E.I. 1997. The performance of male turkeys fed a starter diet containing a mannan oligosachharide. *Zootecnica International*, **20**: 30–32.
Schrezenmeir, J. and De Vrese, M. 2001. Probiotics, prebiotics and synbiotics- approaching a definition. *American Journal of Clinical Nutrition*, **73**: 361–364.
Sczerbinska, D., Tarasewicz, Z., Danczak, A. and Ligocki, M. 2000. The response of growing quails to diets containing oligosaccharides isolated from seeds of narrow-leaved lupin. *Journal of Animal and Feed Sciences*, **9**: 505–512.
Shao, L.P., Zhou,L.J., Li, G.P. and Lin, I.P. 2000. Effects of different levels of mannan-oligosaccharides on cellular immune response and gastro-intestinal microecology in chickens. *Journal of Fujian Agricultural University*, **28**: 86–89.
Smiricky-Tjardes, M.R., Grieshop, C.M., Flickinger, E.A., Bauer, L.L. and Fahey, G.C. 2003. Dietary galactooligosaccharides affect ileal and total-tract nutrient digestability, ileal and fecal bacterial concentrations, and ileal fermentative characteristics of growing pigs. *Journal of Animal Science*, **81**: 2535–2545.
Stanley, V.G. and Sefton, A.E. 1999. Egg and serum cholesterol as influenced by mannanoligosaccharide and aflatoxin. In:Egg nutrition and biotechnology (Ed. Sin, J.S., Nakai, S., Guenter, W.) Walling ford, U.K., CABI Publishing pp. 441–443.
Swamy, H.V.L.N. and Devegowda, G. 1998. Ability of mycosorb to counteract aflatoxicosis in commercial broilers. *Indian Journal of Poultry Science*, **33**: 273–278.
Tarasewicz, Z. 1998. Biological assessment of oligosaccharides isolated from Emir variety lupin (Lupinus Angustifolius) seeds in feeding of a reproductive quail stock. *Akademia Rolniczaw Szczecinie* pp. 58.
Terada, A., Hara, H., Sakamoto, J., Sato, N., Takagi, S., Mitsuoka, T., Mino, R., Hara, K., Fujimori, I. and Yamada, T. 1994. Effects of dietary supplementation with lactosucrose (4G-beta-D-galactosylsucrose) on cecal flora, cecal metabolites and

performance in broiler chickens. *Poultry Science*, **73**: 1663–1672.

Tzortzis, G., Goulas, A.K., Gee, J.M. and Gibson, G.R., 2005. A novel galactooligosaccharide mixture increases the bifidobacterial population numbers in a continuous in vitro fermentation system and in the proximal colonic contents of pigs in vivo. *Journal of Nutrition*, **135**: 1726–1731.

Zdunczyk, Z., Juskiewicz, J., Jankowski, J., Biedrzycka, E. and Koncicki, A. 2005. Metabolic response of the gastrointestinal tract of turkeys to diets with different levels of mannan-oligosaccharide. *Poultry Science*, **84**: 903–909.

5 Enzymes

Feed accounts for a significant cost of production in livestock and poultry. In case of poultry, it represents 65% cost of the production in broilers and 75% in layers. Presently the live stock industry faces a huge challenge due to the high cost of feed ingredients and shortage of the conventional feed ingredients. Over the years, efforts have been made to use unconventional feed ingredients. However, such feed ingredients have many anti nutritional factors that adversely affect the performance of livestock and poultry. Due to the presence of these incriminating factors, the unconventional feed ingredients are poorly digested in livestock and poultry. These poorly digested feed ingredients results in bacterial overgrowth, which becomes a potential substrate for bacterial fermentation leading to intestinal disorders and disease. Application of antibiotics and other anti-microbials help to overcome the ill effects. However, the ban on the use of sub therapeutic level of antibiotics in many countries has increased the relevance of use of exogenous enzymes in the feed of livestock and poultry. These exogenous feed enzymes improve the nutritive value of different feed stuffs, economize livestock and poultry rearing and reduce environmental pollution.

Enzymes are organic and biological catalysts that can initiate or accelerate biochemical reactions, converting one or more substrate into products. In other words, enzymes are protein molecules that catalyze specific chemical reactions. Several exogenous enzymes have been used in livestock and poultry to optimize the production. Besides the auto enzymatic digestion, ruminants have an added advantage of alloenzymatic digestion provided by rumen microflora, which helps to obtain nutrients from complex feed unlike poultry and other monogastric animals (Pariza and Cook, 2010). Hence, use of exogenous enzymes in poultry and other monogastric animals holds significance from commercial point of view. Thus, several digestive enzymes have been studied for use as additives to enhance animal performance with success in poultry and swine diets.

Poultry

Commercial exploitation of exogenous enzymes started a century back with patenting the process for production of alpha amylase from the fungus *Aspergillus oryzae* (Pariza and Cook, 2010). Presently, most of the enzymes used in the food and beverage industry are from Aspergillus except cellulase and hemicellulase derived from *Trichoderma*. Several enzymes have been exploited or have a potential to be exploited in the poultry feed industry *viz*. phytases, proteases, lipases, cellulose (â-glucanases), galactosidases, xylanases and associated enzymes (Table 5.1). However, the success of an enzyme depends on various factors *viz*. the type of feed, anti nutritive factor in the feed, concentration and spectrum of an enzyme, type of bird, age of the birds etc.

Feed accounts for 65% of the cost of production in broilers and 75% in layers. Hence, optimum utilization of feed is very pivotal for the best results in production economics. There are a lot of anti nutritive factors in poultry feed which minimizes the proper utilization of feed. Supplementation of exogenous enzymes have been tried to counter act the adverse effect of the anti nutritive factors.

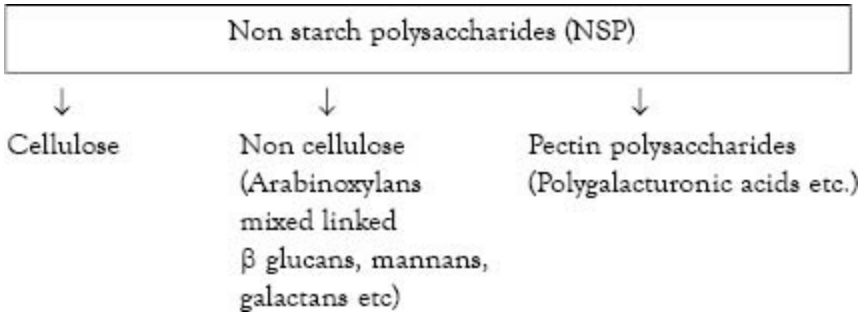
Phytates

Almost two thirds of the total phosphorous found in poultry feed of vegetable origin are found in the form of phytic acid. Phytic acid is the hexaphosphate ester of myo-inositol. As birds lack phytase enzyme, phytates (calcium-magnesium-potassium salt) or phytic acid is poorly utilized by birds and exceted by faeces. Hence, the available phosphorous or non phytin phosphorous is the difference between total and unavailable or phytate phosphorous. It is calculated as plant P X0.30 + supplemental P + P from animal feed origin. Thus, addition of exogenous phytase (*Aspergiulls niger* derived phytase feed enzyme) not only allows intestinal hydrolysis of phytate and thereby increases the availability of phosphorous in feed but also reduces environmental pollution (Pariza and Cook, 2010).

Non starch polysaccharides (NSP)

The classification of non starch polysaccharides was initially based on the method used for extraction and isolation of polysaccharides. The residue left after a series of alkaline extractions of cell wall materials was named cellulose and the fraction of this residue that was solubilised by alkali was called hemicellulose. However, classification by differences in solubility lacks precision with respect to both structure and function. Crude fibre (CF) refers to the residues of plant material after extraction with acid and alkali and includes variable portions of the insoluble NSP. Neutral detergent fibre (NDF) refers to the insoluble portion of the NSP plus lignin, and acid detergent fibre (ADF) refers to a portion of insoluble NSP comprised largely, but not exclusively, of cellulose and lignin. Thus, the nutritional relevance of values obtained using these methods in monogastric nutrition are debatable. The complexity in the structure, function and confusion in the nomenclature has made it almost impossible to draw a precise classification of NSP. Practically, NSP may be classified into three main categories as shown below, namely cellulose, non-cellulosic polymers and pectic polysaccharides (Bailey, 1973).

Non starch polysaccharides (NSP)



The NSP compounds are present in a variety of poultry feed stuffs viz. β glucans in barely and oat, arabinoxylans in wheat and maize, arabinogalactans in rapeseed, galactomannans in guar, galactouronans and galactoarabinans in soybeans. These compounds increase the viscosity, which reduce diffusion and contact with lipase and bile salt micelles. NSP decrease the digestibility of carbohydrates, lipids and protein in poultry. NSP also increase the excreta volume and cause wet litter condition. Thus, a variety of enzymes have been tried to counteract the toxic effects of NSP in poultry as shown in Table 5.1.

Table 5.1. Enzymes used in poultry feed

Enzyme	Classification	Production organism	Function
Phytase	Phosphatase	*Aspergillus* sp., *Penicillium* sp., *Phytase canola*, *Pichia pastoris*, *d- Aspergillus niger*, *d-Escherichia coli*	Hydrolyzes phytate
Glucose oxidase	Oxidoreductase	*Aspergillus niger*	Degrades glucose to hydrogen peroxide and gluconic acid
Catalase	Oxidoreductase	*Aspergillus niger var.*, *Micrococcus lysodeikticus*	Produces water and oxygen from hydrogen peroxide
Trypsin	Protease	Animal pancreas	Hydrolyzes proteins
Protease	Protease	*Aspergillus niger*, *Aspergillus sp.*, *Bacillus* sp.	Hydrolyzes proteins
Pepsin	Protease	Animal stomachs	Hydrolyzes

			proteins
Papain	Protease	Papaya (*Carica papaya*)	Hydrolyzes proteins
Keratinase	Protease	*Bacillus licheniformis*	Hydrolyzes proteins
Ficain	Protease	*Ficus glabrata*	Hydrolyzes proteins
Bromelain	Protease	Pineapple (*Ananas cosmosus*) stem and fruit	Hydrolyzes proteins
Lipase	Lipase	*Aspergillus niger*, *Candida* sp., *Rhizomucor* sp., *Rhizopus* sp.	Hydrolyze triglycerides, diglycerides and glycerol monoesters
Xylanase	Carbohydrase	*Aspergillus* sp., *Bacillus* sp., *Humicola* sp., *Penicillum* sp., *Trichoderma*	Hydrolyzes xylans
Pullulanase	Carbohydrase	*Bacillus acidopullulyticus*	Hydrolyzes starch
Pectinase	Carbohydrase	*Aspergillus aculeatus*, *Aspergillus niger*, *Rhizopus oryzae*	Breaks down protein
β-Mannanase	Carbohydrase	*Aspergillus niger*, *Bacillus lentus Trichoderma reeseic*	Hydrolyzes beta-mannans
Lactase	Carbohydrase	*Aspergillus niger*, *Aspergillus oryzae*, *Candida pseudotropicalis*	Hydrolyzes lactose to glucose and galactose
Invertase	Carbohydrase	*Aspergillus niger*, *Saccharomyces* sp.	Hydrolyzes sucrose to glucose and fructose
Hemicellulase	Carbohydrase	*Aspergillus* sp., *Bacillus* sp., *Humicola* sp., *Trichoderma* sp	Breaks down hemicellulose
Glucoamylase (amylosglucosidase)	Carbohydrase	*Aspergillus niger*, *Aspergillus oryzae*, *Rhizopus niveus*, *Rhizopus oryzae*	Hydrolyzes starch with production of glucose
β-Glucosidase	Carbohydrase	*Aspergillus niger*	Hydrolyzes cellulose degradation products to

β-Glucanase	Carbohydrase	*Aspergillus* sp., *Bacillus* sp.	glucose Hydrolyzes β-glucans
α-Galactosidase	Carbohydrase	*Aspergillus niger*, *Morteirella vinaceae var*	Hydrolyzes oligosaccharides *Saccharomyces* sp.
Cellulase	Carbohydrase	*Aspergillus niger*	Breaks down cellulose
α-Amylase	Carbohydrase	Barley malt	Hydrolyzes starch with production of maltose
Maltogenic α-amylase	Carbohydrase	*Bacillus subtilis*, *d-Bacillus stearothermophilus*	Hydrolyzes starch with production of maltose
α-Amylase	Carbohydrase	*Aspergillus* sp., *Bacillus* sp., *Rhizopus* sp.	Hydrolyzes starch

(Munir and Maqsood, 2013)

In the last century, use of enzymes in poultry feed took a new direction when it was reported that newly hatched chicks may be deficient in digestive enzymes (Nitsan *et al*., 1991; Noy and Sklan, 1995 and Jin *et al*., 1998). Specific activities of lipase, amylase and trypsin rapidly increase up to 2-3 weeks post hatch. Further, it has been suggested that the immaturity of the digestive system of neonates may result in poor utilization of dietary nutrients (Jin *et al*., 1998). In addition, It has also been demonstrated that nutrient digestion rather than the ability to absorb nutrients may be the primary limiting factor (Parsons, 2004). Therefore, dietary supplementation of microbial lipase, amylase or protease enzymes not produced in sufficient quantities by chickens in juvenile stage have been carried out with no significant improvement in body weight gain and FCR (Slominski *et al*., 2006).

Phytase

However, feeding enzyme preparations to improve ruminal digestion has been a questionable practice in the past. The reasoning behind this thought came from the fact that enzymes are proteins and they would be subject to degradation by microbial proteases in the rumen and/or inactivated by proteases in the small intestine.

Adding Enzymes (dry) to Animal Feed

Kopecny *et al*. (1987) reported that a cellulase enzyme complex was rapidly degraded by rumen bacterial proteases and addition to ruminal fluid had no effect on *in vitro* fiber digestion. Some have suggested that feeding unprotected enzymes may be more useful in immature ruminants where rumen microbial populations are not fully developed. For example, Baran and Kmet (1987) reported that a pectinase-cellulase enzyme additive improved ruminal fermentation in newly weaned lambs but not in adult sheep (with established rumen microflora). Recently, there has been renewed interest in the use of enzymes in ruminant diets because some fibrolytic enzymes have been shown to be stable when incubated with protease enzymes. Fontes *et al*. (1995) reported that several xylanases were resistant to several proteases but only one cellulase from a mesophilic organism was resistant to proteolytic attack. Posttranslational glycosylation has also been reported to protect enzymes from deactivation caused by high temperatures and proteinases (Olsen and Thomsen, 1991). Hirstov *et al*. (1998) reported that when added to the rumen, fibrolytic enzymes maintained partial activity. However, integrity of the enzyme is not the only criteria that should be used when evaluating enzymes for ruminant diets, because in order for them to be effective, they must bind to their substrate and catalyze reactions. Tricarico and Dawson (1999) reported that the addition of xylanase and cellulase enzyme preparations improved the in vitro ruminal digestion of fescue hay. Zinn and Salinas (1999) reported that a rumen-stable fibrolytic enzyme supplement increased the ruminal digestion of NDF and Feed N by 23 and 5%, respectively. They also reported an improvement in dry matter intake and average daily gain in steers

supplemented with this additive. These data suggest that adding enzymes directly (in a dry form) to the diets of ruminants may improve digestion and production.

Spraying Enzymes (liquid) Directly onto Animal Feeds

In the past use of enzymes was restricted to their application on to forages at the time of ensiling. However, this mode of application has met with variable results. One method to protect or minimize enzyme degradation by ruminal proteases is to treat feeds with enzymes just prior to feeding. When enzymes are applied to feed in this fashion, binding with substrates can cause conformational changes that may help to protect these exogenous enzymes from ruminal degradation. Treacher and Hunt (1996) reviewed the use of spraying enzymes directly onto feeds, rather than adding at the time of ensiling, to enhance their nutritive values. This approach offers exciting possibilities for using enzymes to improve nutrient digestion, utilization, and productivity in ruminants and at the same time reduce animal fecal material and pollution. Spraying enzymes onto feeds just before feeding provides increased management flexibility for feeding and bypasses any negative interactions that the ensiling process may have on enzyme performance. A number of different mechanisms have been theorized as reasons for positive effects including, direct hydrolysis, improvements in palatability, changes in gut viscosity, complementary actions with ruminal enzymes, and changes in the site of digestion (Beauchemin and Rode, 1996; Treacher and Hunt, 1997). Feng *et al*. (1992) reported that pretreatment of dry grass with fibrolytic enzymes improved in vitro ruminal fiber digestion. Lewis *et al*. (1996) reported that enzymes sprayed onto a grass hay: barley diet increased VFA production and NDF digestion. Spraying enzymes on silage has increased the release of residual sugars and rate of NDF digestion. A growing body of evidence exists that supports improvements in animal productivity when feeds are treated with enzymes prior to feeding. In some instances, enzymes have been applied directly to the grain but in some studies enzymes were applied only to the forage component of the diets prior to mixing into a TMR. Interestingly, several, but not all, publications have reported that high levels of enzymes resulted in lower milk yields than moderate levels of enzyme treatment (Lewis *et al*., 1999; Beauchemin *et al*., 1995, Kung *et al*., 2000). Over-treatment of feeds with enzymes may result in blocking sites that may otherwise be available for microbial enzymatic digestion or may prevent attachment by rumen microbes. More research will be needed in this area.

Evaluating the activity of enzyme additives and predicting improvement in animal performance will be a challenge for future research because temperature, time, substrate concentration, enzyme concentration, product reactions, cofactors, and pH, among other factors, have profound effects on enzyme activity. In addition, sources (bacterial versus fungal) and activity of enzymes differs markedly. The purity of enzyme products must also be ascertained because many commercial enzymes are actually complexes of various enzymes that must work in concert to hydrolyze a substrate to monomer units. For example, crude preparations of a cellulase enzyme complex actually contain endo- and exo-beta-1, 4 glucanases, beta-glucosidases, and cellobiase. Hemicellulase preparations are even more complex. Determining the proper ratio of individual enzyme activities relative to the targeted feed must be determined in order to optimize their effects on feeds. No universally accepted methods exist for determining enzyme activity but they are usually based on release of a monomer under optimal and standardized conditions. Certainly, newer methods that evaluate enzymes should consider their optimum activities at rumen temperature and pH.

We know very little about the stability of added enzymes and interactions of enzymes with components of feeds. If added during processing, enzymes must be able to withstand temperatures during pelleting. Several practical problems must be addressed before liquid enzymes will find acceptance on the farm. First, liquid enzymes will probably require refrigeration for prolonged storage and thus bulk storage space will be needed. In addition, sprayer mechanisms must be mounted on to TMR wagons. The cost of transporting liquid enzymes to farms will also be high because of the weight of the liquid.

References

Bailey, R.W. 1973. Structural Carbohydrates. In: Butler G.W. and Bailey R.W., eds., Chemistry and Biochemistry of Herbage. New York: Academic Press, **1:**157-211.

Baran, M. and Kmet, V. 1987. Effect of pectinase on rumen fermentation in sheep and lambs. *Archives of Animal Nutrition*. **7/8:** 643.

Beauchemin, K. A., Rode, L.M. and Sewalt, V.J.H. 1995. Fibrolytic enzymes increase fiber digestibility and growth rate of steers fed dry forages. *Canadian Journal of Animal Science*, **75**: 641-644.

Beauchemin, K. A. and Rode, L.M. 1996. Use of feed enzymes in ruminant nutrition. *Proceedings of the Canadian Society of Animal Science Annual Meeting*, Lethbridge, Alberta. 103-140.

Beauchemin, K. A., Yang, W.Z. and Rode, L.M. 1999. Effects of grain source and enzyme additive on site and extent of nutrient digestion in dairy cows. *Journal of Dairy Science*, **82**: 378-390.

Feng, P., Hunt, C.W., Julien, W.E., Dickinson, K. and Moen, T. 1992. Effect of enzyme addition on *in situ* and *in vitro* degradation of mature cool-season grass forage. *Journal of Animal Science*, **70** (Suppl. 1): 309 (Abstract).

Fontes, C.M. G. A., Hall, J., Hirst, B.H., Hazelwood, G.P. and Gilbert, H. J. 1995. The resistance of cellulases and xylanases to

proteolytic inactivation. *Applied Microbiology and Biotechnology*, **43**: 52-57.
Fuller, R. 1989. Probiotics in man and animals. *Journal of Applied Bacteriology*, **66**: 365-378.
Hirstov, A., McAllister, T. A. and Cheng, K. J. 1998. Effect of dietary or abomasal supplementation of exogenous polysaccharide-degrading enzymes on rumen fermentation and nutrient digestibility. *Journal of Animal Science*, **76**: 3146-3156.
Jin, S. H., Corless, A. and Sell, J. L. 1998. Digestive system development in post-hatch poultry. *World's Poultry Science Journal* , **54**: 335-345.
Kopency, J., Marounek, M. and Holub, K. 1987. Testing the suitability of the addition of *Trichoderma viride cellulases* to feed rations for ruminants. *Zivocisna Vyroba*, **32**: 587.
Kung, L., Jr., Treacher, R. J., Nauman, G. A., Smagala, A.M., Endres, K.M. and Cohen, M. A. 2000. The effect of treating forages with fibrolytic enzymes on its nutritive value and lactation performance of dairy cows. *Journal of Dairy Science*, **83**: 115-122.
Lewis, G. E., Hunt, C.W., Sanchez, W. K., Treacher, R., Pritchard, G. T. and Feng, P. 1996. Effect of direct-fed fibrolytic enzymes on the digestive characteristics of a forage-based diet fed to beef steers. *Journal of Animal Science*, **74**: 3020-3028.
Lewis, G. E., Sanchez, W. K., Hunt, C.W., Guy, M. A., Prichard, G.T., Swanson, B. I. and Treacher, R.J. 1999. Effect of direct-fed fibrolytic enzymes on the lactational performance of dairy cows. *Journal of Dairy Science*, **82**: 611-617.
Munir, K. and Maqsood, S. 2013. A review on role of exogenous enzyme supplementation in poultry production. *Emirates Journal of Food and Agriculture*, **25**: 66-80.
Nitsan, Z., Duntington, E.A. and Siegel, P.B. 1991. Organ growth and digestive enzyme levels to fifteen days of age in lines of chickens differing in body weight. *Poultry Science*, **70:** 2040-2048.
Noy, Y. and Sklan. D. 1995. Digestion and absorption in the chick. *Poultry Science*, **74:**366-373.
Olsen, O. and Thomsen, K. K. 1991. Improvement of bacterial b-glucanase thermostability by glycosylation. *Journal of General Microbiology*, **137**: 579-585.
Orr, C. L., Ware, D. R., Manfredi, E. T. and Hutchenson, D. P. 1988. The effect of continuous feeding of *Lactobacillus acidophilus* strain BT1386 on gain and feed efficiency of feeder calves. *Journal of Animal Science*, **66** (Suppl. 1): 460 (Abstract).
Pariza, M.W. and Cook, M. 2010. Determining the safety of enzymes used in animal feed. *Regulatory Toxicology and Pharmacology*, **56**: 332-342.
Parsons, C.M. 2004. Gastrointestinal development and nutrient digestion in chicks, in: *Proceedings of the 25th Western Nutrition Conference*, Saskatoon, Canada, 169-176.
Rode, L. M., Yanf, W. Z. and Beauchemin, K. A. 1999. Fibrolytic enzyme supplements for dairy cows in early lactation. *Journal of Dairy Science*, **82:** 2121-2126.
Schingoethe, D. J., Stegeman, G. A. and Treacher, R. J. 1999. Response of lactating dairy cows to a cellulase and xylanase enzyme mixture applied to forages at the time of feeding. *Journal of Dairy Science*, **82:** 996-1003.
Slominski, B.A., Meng, X., Jia, W., Guenter, W. and Jones, O. 2006. The effect of lipase, amylase and protease addition on growth performance and nutrient digestion in young broiler chickens fed corn-soybean meal diet. *Proceedings of the 12th European Poultry Conference*, Sept. 10-14, Verona, Italy (CD ROM).
Treacher, R. J. and Hunt, C.W. 1996. Recent developments in feed enzymes for ruminants. *Proceedings of Pacific Northwest Nutrition Conference*. Seattle, WA.
Tricarico, J. M. and Dawson, K. A. 1999. Effects of defined xylanase and cellulase enzyme preparations on digestive processes of ruminal microbial cultures. *Journal of Dairy Science*, **77** (Suppl. 1): 252. (Abstract)
Yang, W. Z., Beauchemin, K. A. and Rode, L. M. 1999. Effects of an enzyme feed additive on extent of digestion and milk production of lactating dairy cows. *Journal of Dairy Science*, **82:** 391 403.
Zinn, R. A. and Salinas, J. 1999. Influence of Fibrozyme on digestive function and growth performance of feedlot steers fed a 78% concentrate growing diet. *Proceedings of Alltech 15th Annual Symposium.* Biotechnology in the Feed Industry. Nottingham University Press. 313-319.

6 Organic Acids

An organic acid is an organic compound with acidic properties. The most common organic acids are the carboxylic acids, whose acidity is associated with their carboxyl group –COOH. However, sulphuric acids, containing the group $–SO_2OH$, are relatively stronger acids. Alcohols, with –OH, may also act as weak acids. Some other groups may also act as weak acids *viz*. thiol group and phenol group. In biological systems, organic compounds containing these groups are generally classified under organic acids. Some common organic acids are lactic acid, acetic acid, formic acid, citric acid, oxalic acid, uric acid, propionic acid and butyric acid. Organic acids have been used for decades in commercial compound feeds, mostly for feed preservation, for which formic and propionic acids are particularly effective. Since the ban of antibiotic growth promoters in many countries, organic acids have been used increasingly, not only because of their preservative qualities, but also for their nutritive properties. Organic acids and their salts are generally regarded as safe and have been approved by most member states of EU to be used as the feed additives in animal production.

Organic acids are both bacteriostatic and bactericidal. As un-dissociated organic acids are lipophilic, they can cross the cell membrane of Gram negative bacteria, such as *Salmonella*. Once inside the cell, the higher cytosolic pH causes the acid to dissociate, releasing hydrogen ions, which consequently reduces the intracellular pH. Microbial metabolism is dependent on enzyme activity, which is depressed at lower pH. To redress the balance, the cell is forced to use energy to expel protons out across the membrane via the H+-ATPase pump to restore the cytoplasmic pH to normal. Over a period of exposure to an organic acid, this can be sufficient to kill cell. Expelling protons also leads to an accumulation of acid anions in the cell (Lambert and Stratford, 1998), which inhibits intracellular metabolic reactions, including the synthesis of macromolecules, and disrupts internal membranes. Lactic acid bacteria are less sensitive to the pH differential across the cell membrane, and thus remain unaffected. Inhibition of microbial growth by this mode of action has been exploited for thousands of years in food preservation; organic acids are natural by-products of microbial metabolism.

Poultry

Organic acids in un-dissociated (non ionised, more lipophilic) form can penetrate the bacteria cell wall and disrupt the normal physiology of certain types of bacteria. Besides antimicrobial activity, they reduce the pH of digesta and increase the pancreatic secretion. Acidification with various organic acids reduces the production of toxic components by the bacteria and colonization of pathogens on the intestinal wall, thus preventing the damage to epithelial cells. Organic acids improve the digestibility of proteins, calcium, phosphorus, magnesium, and zinc. Thus, they enhance nutrient utilization leading to better growth and feed conversion efficiency.

Organic acid are lipid soluble in the un-dissociated form in which they are able to enter the microbial call. However carrier mediated transport mechanism seem to be also involved in the membrane transportation.

Butyric acid

Butyric acid may improve growth, feed efficiency and GIT health. Butyric acid given to broiler chicks may influence body weight gain and feed conversion ratio. It has been observed that pH of gastro intestinal tract (GIT) is reduced by butyric acid supplementation (Leeson *et al*., 2005).

Butyric acid may improve dressing percentage and reduce abdominal fat in commercial broilers. Further, butyric acid may play a role in the development of intestinal epithelium (Rama Rao *et al*., 2003).

Acetic acid

Acetic acid improves the heath of chicken due to higher production of pancreatic enzyme, which improves GIT health. Dietary inclusion of acetic acid may improve FCR, bodyweight and carcass yield in broilers (Denli *et al*., 2003; Abdel Fattah *et al*., 2008).

Citric acid

Citric acid reduces PH of digesta, may improve gut health and degrades aflatoxin in the poultry ration. In addition, citric acid may also improve poultry performance by reducing colonization of pathogenic micro-organism and toxic bacterial metabolites such as ammonia and amines. Citric acid improves F.C.R, gut health immunity and live body weight in broilers (Chaveerach *et al*., 2004; Mendez Albores *et al*., 2005).

Propionic acid

Propionic acid may affect the integrity of microbial cell membrane or cell macro molecules or interfere with nutrient transport and energy metabolism causing the bactericidal affect. Propionic acid has PH reducing property. It also improves gut health, FCR, immunity in broiler chicks at young stage (Dibner and Buttin, 2002).

Lactic acid

Lactic acid bacteria break down lactose (milk sugar) into glucose and galactose. The acidic environment inhibits the growth of harmful bacteria. Lactic acid bacteria plays a role in absorption of vitamins D and K and formation of soluble salts of calcium and iron in broilers (Schingoethe, 1976 and Morishita *et al*., 1982).

Role of organic acid in egg production and quality parameters

Phosphorus (P) is an essential mineral for metabolism and egg production of layers. It has been observed that organic acid supplementation at 780 ppm maintained production end till 70 weeks of age in layers. Organic acid diet increases egg shell quality, egg shell protein, egg shell calcium, improve mineral and protein absorption. It has also been reported that organic acid in diet increases hen day egg production and hen house egg production in layers (Zeidler, 2001; Rodriguez Navarro *et al*., 2002 and Chen and Chen, 2004).

Role of organic acid in immunity of poultry

The first 3 weeks of life comprise a critical period in chick's life for gut maturation. During this period healthy birds perform at maximum potential without diverting energy towards immune response processes (Klasing, 2007; Yegani and Korver, 2008 and Yin *et al*., 2010). Gut morphology affects nutrient absorption, and greater villi length and crypt depth are associated with functional ability (Stokes *et al*., 2001; Yang *et al*., 2007 and Choct, 2009). Commensal bacteria of the gut benefit the host in a variety of ways, including organic acid production and immunomodulation (Tse and Chadee, 1991). Organic acids inhibit pathogenic bacteria growth by disrupting bacteria cell membrane transport, preventing the bacteria from reaching equilibrium with their environment (Cherrington *et al*., 1990). Probiotics, beneficial microbial cultures, may be administered to stimulate the local immune system (Fuller, 1989; Gibson and Roberfroid, 1995; Netherwood *et al*., 1999) and enhance epithelial innate immunity-related gene expression through anti-inflammatory effects and reduced pro-inflammatory cytokine expression such as IL-6 (Pagnini *et al*., 2010).Therefore, the organic acids pay a pivotal role on intestinal morphology and innate immunological responses of chickens during this period. It has been reported that level of gamma globulin increased in broilers with dietary inclusion of organic acids (Rahmani and Speer, 2005).

Pigs

Addition of organic acids to feed combats susceptible microorganisms, including pathogenic bacteria and some fungi, which would otherwise cause spoilage and reduce its nutritive value by metabolizing the starch and protein therein. In pig diets, organic acids and their salts also take effect in the gastrointestinal tract, mainly in the proximal tract - the stomach and small intestine. Firstly, organic acids lower the pH of the stomach contents, which can especially beneficial at weaning, where it stimulates the conversion of inactive pepsinogen to active pepsin. This may improve protein digestibility and decrease the rate of gastric emptying. Organic acids also stimulate exocrine pancreatic secretion of enzymes and bicarbonate, thus assisting with protein and fat digestion. Furthermore, organic acid anions can complex with calcium, phosphorus, magnesium and zinc, improving the digestion of these minerals and

reducing the excretion of supplemental minerals and nitrogen (Roth *et al*., 1998a, b). The bacteriostatic or bactericidal effects of organic acid anions also take effect in the proximal gastrointestinal tract. It should be noted that whereas organic acids lower gastric pH, organic acid salts do not (Eidelsburger *et al*., 1992a). Therefore, the improvements in growth performance resulting from dietary inclusion of organic acid salts are due to an antimicrobial effect. Butyric acid for instance, is the main energy source for the epithelial cells of the large intestine and is considered to be effective for promoting epithelial growth (Galfi and Bokori, 1990).

Organic acid on pig performance

At weaning, piglets are particularly susceptible to infection with intestinal pathogens, as well as being inadequately equipped physiologically to deal with solid feed. The buffering capacity of weaning feeds is also high, compounding the problem through a negative effect on pepsin activity in the stomach (Eidelsburger *et al*., 1992b), a problem that is addressed through the acidification of diet. In the diet of grower finisher pigs, the anti-microbial effect of organic acids in the feed (hygiene), stomach and small intestine is largely responsible for their performance enhancing benefits. Effective doses have been established that can improve productivity of pigs to levels comparable with antibiotic growth promoters (Øverland *et al*., 2000).

Most recently it has been studied adding organic acids to diets for sows. Øverland *et al*. (2009) added 0.8% or 1.2% potassium diformate to diets for primiparous and multiparous sows from one mating to ting of acidifiers the next, feeding of acidifier through gestation and lactation. The performance of the piglets of these sows was also recorded and compared. The authors found that sows fed potassium diformate had increased back fat thickness during gestation, although daily feed intake and body weight gain did not change. Feeding potassium diformate also tended to be associated with a heavier birth weight of piglets, irrespective of dose. It also improved average daily gain, resulting in a greater weaning weight. Sows fed the diets containing potassium diformate tended to have increased milk fat content on day 12 post-farrowing. On the other hand, sows fed potassium diformate at a dosage of 0.8% under tropical conditions (Lückstädt, 2011) tended ($P < 0.1$) to have a higher feed intake from 3 days after farrowing onwards. Furthermore, reduced weight loss ($P = 0.06$) during the weaning period and lower back fat loss ($P = 0.05$) was observed.

Salmonella control in fattening pigs

Good gut health is increasingly being shown to be effective against intestinal pathogens, a strategy that has only been made possible through the removal of antibiotic growth promoters in feed. Creating and maintaining a healthy intestinal environment has become essential to productivity and food safety programmes alike. *Salmonella enteritica Typhimurium* is the predominant serotype found in pig carcasses, accounting for about 71% of cases. Several serotypes are resistant to antibiotics, which has put pressure on producers to prevent contamination. While salmonella cannot be wholly eradicated in pig units, it can be controlled to minimise the risk to consumers. Biosecurity plays a significant role in salmonella control. In feed compounding, although heat treatment is effective in reducing contamination of feed leaving the feed mill, this effect does not persist during transport, storage and subsequent outfeeding. When conditions within the feed are less conducive to bacterial infection, salmonella contamination can be reduced. The next critical control point is within the pig's gut, where conditions for bacterial growth may again be optimal. Salmonella growth requires warmth (35- 370 C is optimal), a moisture content greater than 12% and a pH between 4.5 and 9.0. It is no coincidence that the pig gut can provide salmonella everything they need to thrive.

A study by Dennis and Blanchard (2004) in the UK and a more recent study by Correge *et al*. (2010) in France also concluded that potassium diformate is an effective tool in salmonella control strategies on commercial farms, as it reduced the percentage of salmonella-positive pigs by 50% and decreased salmonella ELISA scores in pork meat juice by 46% in grower finisher pigs. The UK trial also showed an improvement in daily gain of 7.7%, reduced mortality and a reduction in medicinal intervention compared with the rolling average for the unit, which showed an economic benefit for implementing salmonella control measures.

Ruminants

Very few researches have been conducted to evaluate the effects of organic acids on ruminant performance. Kung *et al*. (1982) reported that feeding 140 g of malate per day increased milk persistency in lactating cows and increased total VFA during early lactation. Feeding malate to Holstein bull calves improved ADG and feed efficiency but had little effect on blood serum constituents (Sanson and Stallcup, 1984). Even though *in vitro* studies have shown that DL-malate favorably alters ruminal fermentation (Martin and Streeter, 1995; Callaway and Martin, 1996), little information is available detailing the effects of DL-malate on beef cattle performance. In another studies, the addition of malic acid into ruminant concentrates is reported to have a positive effect on daily live weight gain (Martin *et al*. 1999; Mungói, 2007a) whereas in others (Carro *et al*. 2006; Mungói, 2007b), no change in the daily weight gain was found. Carro *et al*. (2006) stated that the addition of malic acid into lamb rations didn't affect forage and concentrate intake, but Mungói (2007b) stated that the forage intake increased and the concentrate intake decreased according to the gradual incorporations of malic acid into concentrates for lambs during the growth period. With the addition of malic acid into ruminant food, the changes in the rumen fermentation products and ruminal pHs are reported to be similar to the effects of

ionophores (Martin and Streeter, 1995). Martin and Streeter (1995) stated that the total volatile fatty acids and the ammonia nitrogen contents in the rumen from cows supplemented with DL malate yielded similar results with not supplemented controls. However, Mungói (2007b) reported that the total volatile fatty acids increased in ruminants supplemented with organic acids.

References

Abdel-Fattah, S.A., El-Sanhoury, M.H., El-Mednay, N.M. and Abdel-Azeem, F. 2008. Thyroid activity, some blood constituents, organs morphology and performance of broiler chicks fed supplemental organic acids. *International Journal of Poultry Science*, **7:**215–222.

Callaway, T. R., and Martin, S. A. 1996. Effects of organic acid and monensin treatment on in vitro mixed ruminal microorganism fermentation of cracked corn. *Journal of Animal Science*, **74**:1982”1989

Carro, M.D., Ranilla, M.J., Giráldez, F.J. and Mantecón, A.R. 2006. Effects of malate on diet digestibility, microbial protein synthesis, plasma metabolites, and performance of growing lambs fed a highconcentrate diet. *Journal of Animal Science*, **84**: 405-410.

Chaveerach, P., Keuzenkamp, D.A., Lipman, L.J.A and Van Knapen, F. 2004. Effect of organic acids in drinking water for young broilers on *Campylobacter* infection, volatile fatty acid production, gut micro flora and histological cell changes. *Poultry Science*, **83**: 330-334.

Chen, Y.C. and Chen, T.C. 2004. Mineral utilization in layers as influenced by dietary oligo fructose and inulin. *International Journal of Poultry Science*, **3**: 442-445.

Cherrington, C. A., Hinton, M. and Chopra, I. 1990. Effect of short-chain organic acids on macromolecular synthesis in *E. coli*. *Journal of Applied Bacteriology*, **68:** 69-74.

Choct M. 2009. Managing gut health through nutrition. *British Poultry Science*, **50***:* 9–15.

Corrégé, I., Le Roux, M., Royer, E. and Rémigereau, O. 2010. Effect of acidifying fattening feed to reduce carriage of Salmonella in high prevalence pig herd. *Journees Recherche Porcine*, 217-218.

Denli, M., Okan, F. and Celik, K. 2003. Effect of dietary probiotic, organic acid and antibiotic supplementation to diets on broiler performance and carcass yield. *Pakistan Journal of Nutrition*, **2**: 89–91.

Dennis, I. and Blanchard, P. 2004. Effect of feeding potassium diformate on incidence of salmonella infection on a commercial unit. *The Pig Journal*, **54**:157-160.

Dibner, J.J. and Buttin, P. 2002. Use of organic acid as a model to study the impact of gut microflora on nutrition and metabolism. *Journal of Applied Poultry Research*, **11**: 453-463.

Eidelsburger, U., Kirchgessner, M. and Roth, F.X. 1992a. Zum einflub von Ameisensaure, Calciumformiat und Natriumhydrogencarbonat auf pH-Wert, Trockenmassegehalt, Konzentrationen an Carbonsäure und Ammoniak in verschiedenen Segmenten des Gastrointestinaltraktes. 8. Mitteilung: Untersuchungen zur nutritiven Wirksamkeit von organichen Säuren in der Ferkelaufzucht. *Journal of Animal Physiology and Animal Nutrition*, **68**: 20-32.

Eidelsburger, U., Kirchgessner, M. and Roth, F.X. 1992b. Zum einflub von Ameisensaure, Calciumformiat und Natriumhydrogencarbonat auf tägliche Zunahmen, Futteraufnahme, Futterverwertung und Verdaulichkeit. 7. Mitteilung. Untersuchungen zur nutritiven Wirksamkeit von organischen Säuren in der Ferkelaufzucht. *Journal of Animal Physiology and Animal Nutrition*, **68**: 258-267.

Fuller, R. 1989. Probiotics in man and animals. *Journal of Applied Bacteriology*, **66***:*365–378.

Galfi, P. and Bokori, J. 1990. Feeeding trial with a diet containing sodium n-butyrate. *Acta Veterinaria Hungarica*, **38**(1-2): 3-17.

Gibson, G.R. and Roberfroid, M.B. 1995. Dietary modulation of the human colonic microflora: introducing the concept of prebiotics. *Journal of Nutrition*, **125***:*1401-1412.

Klasing, K.C. 2007. Nutrition and the immune system. *British Poultry Science*, **48***:*525-537.

Kung, L., Jr., Huber, J.T., Krummrey, J.D., Allison, L. and Cook, R.M. 1982. Influence of adding malic acid to dairy cattle rations on milk production, rumen volatile acids, digestibility, and nitrogen utilization. *Journal of Dairy Science*, **65**:1170-1174.

Lambert, R.J. and Stratford, M, 1998. Weak-acid preservatives: modelling microbial inhibition and response. *Journal of Applied Bacteriology*, **86**: 157-164.

Leeson. S., Namkung,H., Antongiovanni, M. and Lee, E.H. 2005. Effect of butyric acid on the performance and carcass yield on broiler chickens. *Poultry Science*, **84**:1418-1422.

Lückstädt, C. 2011. Effects of dietary potassium diformate on feed intake, weight loss and backfat reduction in sows: pre-farrowing till weaning. *Advances in Animal Biosciences*, **2**:145.

Martin, S.A. and Streeter, M.N. 1995. Effect of malate on *in vitro* mixed ruminal microorganism fermentation. *Journal of Animal Science*, **73**: 2141-2145.

Martin, S.A., Streeter, M.N., Nisbet, D.J., Hill, G.M. and Williams, S.E. 1999. Effects of DL-malate on ruminal metabolism and performance of cattle fed a high-concentrate diet. *Journal of Animal Science*, **77**: 1008-1015.

Mendez- Albores, A., Arambula-Villa, G., Loarca-Pina, M.G.F, Castano-Tostado, E. and Moreno-Martinez, E. 2005. Safety and efficiency evaluation of aqueous citric acid to degrade b- aflatoxins in maize. *Food and Chemical Toxicology*, **43**: 233-238.

Morishita, Y., Fuller, R. and Coates, M. E. 1982. Influence of dietary lactose on the gut flora of chicks. *British Poultry Science*, **23:** 349-359.

Mungói, M.A.T. 2007a. Effect of malate and starch source on growth performance, rumen traits, digestibility and nutrient balance of fattening lambs (Capítulo 4). In: Efectos de la suplementación con malato y de la fuente de almidón sobre el rendimiento productivo de corderos de cebo. Tesis Doctoral, Barcelona, pp.: 67-90.

Mungói, M.A.T. 2007b. Effect of increasing malate level on performance, ruminal pH and fermentation profile of growing lambs fed a highconcentrate diet (Capítulo 5). In: Efectos de la suplementación con malato y de la fuente de almidón sobre el rendimiento productivo de corderos de cebo. Tesis Doctoral, Barcelona, pp.: 102-127.

Netherwood, T., Bowden, R., Harrison, P., O'Donnell, A. G., Parker, D.S. and Gilbert, H. J. 1999. Gene transfer in the gastrointestinal tract. *Applied Environmental Microbiol*ogy, **65***:* 5139-5141.

Øverland, M., Bikker, P. and Fledderus, J. 2009. Potassium diformate in the diet of reproducing sows: Effect on performance of sows and litters. *Livestock Science*, **122**: 241-247.

Øverland, M., Mroz, Z., Granli, T. and Stein, S.H. 2000. Performance and mode of action of dietary potassium diformate for weanling pigs. 51st Annual Meeting of the EAAP, The Hague, Netherlands, August, 21-24.

Pagnini C., Saeed R., Bamias G., Arseneau K.O., Pizarro T.T. and Cominelli F. 2010. Probiotics promote gut health through stimulation of epithelial innate immunity. *Proceedings of the National Academy of Sciences of the United States of America*, **107***:* 454–459.

Rahmani, H.R. and Speer, W. 2005. Natural additives influence the performance and humoral immunity of broilers. *International Journal of Poultry Science*, 4: 713-717.

Rama Rao, S.V., Panda, A.K., Raju, M.V.L.N., Sunder, G.S. and Praharaj, N.K. 2003. Requirement of calcium for commercial broiler and white leghorn layer at low dietary phosphorous levels. *Animal Feed Science and Technology*, **106**: 199-208.

Rodriguez-Navarro, A., Kalin, O., Nys, Y. and Garcia-Ruiz, J.M. 2002. Influence of the microstructure on the shell strength of eggs laid by hens of different ages. *British Poultry Science*, **43**: 395-403.

Roth, F.X., Windisch, W. and Kirchgessner, M. 1998a. Effect of potassium diformate (Formi®LHS) on nitrogen metabolism and nutrient digestibility in piglets at graded dietary lysine levels. *Agribiological Research*, **51**:167-175.

Roth, F.X., Windisch, W. and Kirchgessner, M. 1998b. Mineral metabolism (P, K, Mg, Zn, Mn, Cu) and piglets supplied with potassium diformate (Formi®LHS). *Agribiological Research*, **51**(2):1-8.

Sanson, D. W. and Stallcup, O. T. 1984. Growth response and serum constituents of Holstein bulls fed malic acid. *Nutrition Reports International*, **30**:1261"1267.

Schingoethe, D. J. 1976. Whey utilization in animal feeding: A summary and evaluation. *Journal of Dairy Science*, **59**: 556-570.

Stokes, H.W., Holmes, A.J., Nield, B.S., Holley, M.P., Nevalainen, K.M., Mabbutt, B.C. and Gillings, M.R. 2001. Gene cassette PCR: sequence-independent recovery of entire genes from environmental DNA. *Applied and Environmental Microbiology,* **67:** 5240–5246.

Tse S.K. and Chadee K. 1991. The interaction between intestinal mucus glycoproteins and enteric infections. *Parasitology Today*, **7:** 163–*1*72.

Yang, Y., Iji, P.A., Kocher, A., Mikkelsen, L.L. and Choct, M. 2007. Effects of mannan oligosaccharide on growth performance, the development of gut microflora, and gut function of broiler chickens raised on new litter. *Journal of Applied Poultry Research*, **16:** 280–288.

Yegani, M. and Korver, D.R. 2008. Factors affecting intestinal health in poultry. *Poultry Science*, **87:** 2052–2063.

Yin Y., Lei F., Zhu L., Li S., Wu Z., Zhang R., Gao G.F., Zhu B. and Wang X. 2010. Exposure of different bacterial inocula to newborn chicken affects gut microbiota development and ileum gene expression. *The ISME Journal*, **4:** 367–*3*76.

Zeidler, G., 2001. Shell Egg Quality and Preservation. 5th Edn. 945-963.

7 Fatty Acids

Fats are rich sources of energy. Fats provide 2.25 times more available enrgy than carbohydrates. Fats also increase the palatability and reduce the dustiness of feed. Fats and fatty acids decrease the heat increment in the body. Fatty acids are also involved in number of physiological functions of the body. Recently, considerable interest has been shown on studies pertaining to dietary supplementation of polyunsaturated fatty acids (PUFA) in animal feed and their role in animal health and production.

Poultry

Nutrition can modulate quantitative and qualitative aspects of the immune response to pathogens. Research on poultry, especially in chickens has elucidated the impact of diet on immunocompetence (Cook, 1991; Koutsos and Klasing, 2001) and the mechanisms that are responsible. Elevated levels of fat-soluble vitamins and omega-6 fatty acids may influence the hatchling's early inflammatory and immune response. The fatty acid composition of diet affects the fatty acid composition of the yolk, which in turn can affect embryonic development and hatchability. Palmitic acid increase yolk oleic acid levels to affect transport across the yolk sac membrane and spare linoleic acid for embryonic growth and hatchability. Adding corn oil, palmitic acid, oleic acid or linoleic acid to a basal diet increased hatchability and decreased late embryonic mortality (Vilchez *et al*., 1992). It has also been observed that maternal dietary lipids alter bone development by influencing organic matrix quality and mineralisation in embryos (Liu *et al*., 2003).

A redirection of nutrient flow to meet the metabolic requirements of an immune response or inflammatory reaction is referred to as homeorhesis (Bauman and Currie, 1980). This is in clear contrast to the homeostatic mechanism of maintaining metabolic equilibrium. In fact it is the need of the hour to study if dietary manipulation can promote disease resistance and immunity. Protective immune responses require a supply of nutrients at the appropriate times and amounts (Humphrey *et al*., 2002). While amino acids are needed as substrates to aid the production of immunoglobulins, lysozymes, complement, cytokines, monocytes, heterophils and clonal proliferation of antigen driven lymphocytes; fatty acids bind to intracellular receptors or modify the release of secondary messengers. PUFA modulates the intercellular communication by regulating the incorporation of fatty acids into cell membranes (Korver and Klasing, 1995, 1997). Linoleic acid is elongated to arachidonic acid and incorporated into cell membranes. Arachdonate on release is converted into prostaglandins, leukotrienes and thromboxanes. Similarly, the n-3 PUFA can be incorporated into cell membrane and on release determines the amount and type of eicosanoids and thus regulates cell communication. Increases in antibody responses to antigens and decreases in mitogen-induced proliferation of lymphocytes are due to modulatory effects of dietary n-3 fatty acids (Fritsche *et al*., 1991; Korner and Klasing, 1997).

Fatty acids have a profound role on the development of immune system. Hamdy *et al*. (2003) reported that supplementation of sunflower oil along with linseed oil in broiler chicks significantly increased differential leukocyte counts and the relative weight of the bursa of fabricius but there were changes in the relative weights of spleen and thymus.

Cheng *et al*. (2003) observed that anti-bovine serum albumin (BSA) antibody titres in laying hens fed fish oil or linseed oil were higher compared to hens fed the control diet. Selvaraj and Cherian (2004) suggested that n-3 fatty acids increased production performances and antibody mediated responses, while n-6 fatty acids and conjugated linoleic acid increased cell mediated responses in broiler birds. However, Friedman and Sklan (1997) found that in turkeys vaccinated against Newcastle, infectious bronchitis and necrotic enteritis, specific antibody response was related quadratically to serum linoleic acid and total n-6 polyunsaturated fatty acid concentration. No correlation was found with linoleic acid or arachdonic acids. Similarly, Fritsche and Cassity (1992) concluded that n-3 dietary fatty acids did not alter the primary or secondary antibody response of broiler chickens to sheep red blood cells. Rather n-3 fatty acids reduced antibody dependent cell cytotoxicity and altered eicosanoid release by chicken immune cells.

In fact, the ratio of n-3 to n-6 PUFA plays an important role in modulating humoral and cell mediated immune response. Wang *et al*. (2000) observed that a linseed oil diet increased the IgG concentration in laying hen serum. Sunflower oil reduced IgY content in egg

yolk. Torki *et al*. (2003) reported that antibody production against sheep red blood cells in chicks fed a diet with a higher ratio of n-3: n-6 PUFA was higher among the dietary groups where as moderate dietary ratio of n-3: n-6 PUFA improved antibody production against Newcastle disease virus. Similarly, Xia *et al*. (2003) found that antibody titers in fish oil and linseed oil supplemented diets were higher than that in laying hens fed corn oil. The proliferation response to concanavalin was lower in laying hens that were fed oils rich in n-3 fatty acids. They also suggested that higher level of n-3 fatty acids could improve immune functions of laying hens. Thus, they concluded that dietary fat source and level had a significant impact on immune responses of laying hens.

Modulation of the magnitude and isotype of antibody responses of poultry to T cell-dependent antigens is affected not only by type of essential fatty acids but also by their source. Higher total antibody and IgG titres to bovine serum albumin were found especially after primary immunization in pullets given the sunflower oil enriched diet. Birds given sunflower oil mounted significantly lower IgM titres to bovine serum albumin after primary and secondary immunization (Parmentier *et al*., 2002).

Sijben (2002) suggested that inclusion of 1-2% of n-3 and inclusion of no more than 3-4% of n-6 in the diet is optimal for antibody responsiveness, enhances T cell reactivity, and possibly improves chicken's disease resistance. Zaki and Hady (1995) concluded that different dietary fat sources have different impact on performance and immune response of broiler chickens. While a mixture of beef tallow and linseed oil (1:1) when fed to male Cobb broiler chicks significantly increased body weight compared with those fed on the fat sources separately, haemagglutinin antibody titre and delayed hypersensitivity reaction (to phytohaemagglutinin-P) values were higher in chickens fed on linseed oil. Though thymus and spleen weights were not affected by the inclusion of different fat sources, bursal weight was significantly higher at 3 and 7 weeks of age in chickens fed on linseed oil.

Ruminants

Milk yields of high-producing dairy cattle demand that energy intake be maximized. Energy intake is not only important during the period of peak milk production but also must be sufficient to maintain persistency of production and support body weight gain following peak milk yield. To increase ration energy density, cereal grains are used to replace forages, but this practice is limited since a certain amount of effective fiber is required to optimize ruminal fermentation, enhance nutrient digestibility, and maintain DM intake. Recent research indicates that low forage diets can be fed if starch digestion is monitored by considering source of starch, processing and particle size of the cereal grain, and nonforage fiber sources are used to dilute starch from the ration (Firkins *et al*., 2001). Because fat is much higher in energy per unit of weight than cereal grains, it is used to increase energy density of diets. Digestion and absorption of fat, suggested feeding guidelines for various fat sources, and the effects of feeding supplemental fat on milk composition will be discussed in this chapter.

Biohydrogenation and Digestion of Fat

Fat digestion begins in the rumen (Figure 7.1). Bacterial lipases, and possibly to a minor extent plant lipases, break down fat into glycerol and fatty acids (FA). Glycerol is used as an energy source by bacteria and is principally converted to propionic acid. The unsaturated FA are biohydrogenated by bacteria, but the bacteria do not use them as an energy source. Most of the FA flowing to the small intestine, where absorption takes place, will be free FA but some diglycerides, monoglycerides, and microbial phospholipids will reach the small intestine. Presence of pacreatic lipase will result in cleavage of the glycerides and phospholipids so the FA can be incorporated into the micelles for absorption. With the nonpolar nature of lipids, bile is very critical for emulsification of the FA (for their incorporation into micelles) so that absorption can take place. The FA are reassembled as phospholipids and glycerides and packaged as very low density lipoproteins (VLDL) and chylomicrons that have an outer protein matrix to aid in the transport of lipids throughout the lympathic system.

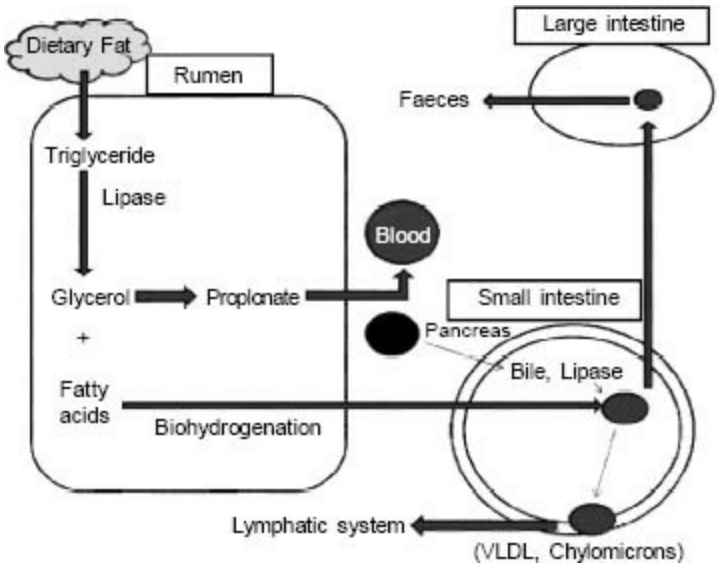


Fig 7.1: Digestion and absorption of fat in ruminants

The desirable characteristics of a fat source are that it should have minimum effects on ruminal fermentation and have a high digestibility; however, these two qualities are not always easy to achieve. Unsaturated fat is highly digestible but may reduce fiber digestibility in the rumen due to inhibitory effects on cellulolytic microorganisms. Saturated fat has less influence on fiber digestion in the rumen, but digestibility of the fat may be inferior depending on the level of saturation (Firkins and Eastridge, 1994; NRC, 2001). When comparing fats based on saturation level, iodine value (IV) or total unsaturates can be used. Iodine value is more reflective of level of unsaturation (or saturation) because the relative proportion of unsaturated fatty acids (e.g. C18:1 versus C18:2 versus C18:3) is not reflected within total unsaturates.

The potential negative impact of unsaturated fat from oilseeds may be minimized if the oilseeds are fed either whole or coarsely cracked rather than extruded (Faldet and Satter, 1991; Reddy *et al*., 1994). This would allow the encapsulated oil to be released at a slower rate in the rumen, or some of the oil may even escape the rumen. Because of fat being nonpolar (hydrophobic), it seeks out particulate matter in the aqueous environment in the rumen, especially attaches to the waxy layer of forages. Free oil from oilseeds should not be added to ruminant diets because of the rapid contact of the oil with particulate matter and microbes. Grinding soybeans should not be done because of reduced rumen undegraded protein in roasted soybeans (Tice *et al*., 1993) and the potential increase in oxidative rancidity with ground raw soybeans (unless grinding is frequent).

Fat Sources

Fat sources can be grouped into two major categories: natural fats and commercial fats. The natural fats can be sub-divided into plant and animal fats. Commercial fats are special preparations made by using animal or plant fats. The chemical and fatty acid compositions of various fat sources are provided in Table 1.

Natural fats

Oilseeds are the major source of fat from plants. Whole cottonseed is a well-balanced feedstuff for dairy cattle because it is relatively high in protein, fiber, and energy. Due to physical characteristics of cottonseed, especially linted cottonseed, they are most easily handled by inclusion in a total-mixed ration. However, the availability of Easiflo (coated with 2.5% starch to mat the linters) and pelleted cottonseed provide for increased potential usage. Whole cottonseed contains the pigment gossypol that is toxic to animals, especially non-ruminants. With the levels of cottonseed typically consumed by dairy cattle (< 15% of DM), intake of sufficient gossypol to cause health problems is not likely (Coppock *et al*., 1987). However, cottonseed should be closely monitored for mycotoxin contamination, especially aflatoxin.

Raw soybeans should not be ground and added to feed mixtures containing urea because soybeans contain urease. Although the trypsin inhibitor in soybeans is a concern when feeding them to non-ruminants, it is assumed that most of the trypsin inhibitor is deactivated in the rumen. On the other hand, some of the trypsin inhibitor may escape ruminal fermentation and decrease crude protein digestibility in the intestines (Palmquist and Conrad, 1971; Tice *et al*., 1993). Soybeans can be included in total-mixed rations, top dressed, or in the case of cracked beans (whole seeds will separate out), can be added to grain mixtures. As alluded to earlier, exposure of oil and release of lipoxygenase by grinding raw soybeans may lead to rancidity problems, especially during summer months. Potential for the oil to become rancid is greater when the ground soybeans are added to ensiled forages or wet by-products during warm months.

Roasting soybeans will denature the urease, trypsin inhibitor, and lipoxygenase and decrease ruminal degradability of the protein. For example, protein degradability in raw soybeans is about 72% whereas protein degradability in roasted soybeans is about 50%. Quality of the roasting can be quite variable from one roaster to another with such factors as temperature, moisture level of seed, rate of transit through roaster, and handling of beans after exiting the roaster giving rise to some of the quality differences. A few beans should be broken and examined for uniformness of heat penetration - beans should not be raw in the center. Over-heating of beans will reduce protein digestibility; therefore, occurrence of charred seeds should be avoided. Although visual appraisal alone is not adequate for assessing quality of the roasting, seeds should be light brown in color. The protein dispersibility index (Hsu and Satter, 1995) is available from some labs as a measure of the adequacy of heat processing of soybeans.

Other oilseeds such as canola, safflower seeds, and sunflower seeds can also be fed to dairy cattle. Caution should be exercised in feeding these oilseeds because the oil is highly unsaturated, and they are higher in oil than cottonseed and soybeans, resulting in lower recommended feeding levels. For example, only about one-half as much canola (seeds should be cracked) can be fed as cottonseed or soybeans.

The primary animal fat fed to dairy cattle is tallow. Tallow contains more saturated fatty acids than the oilseeds, but handling is more difficult because it is solid or semi-solid at room temperature. Tallow can be readily purchased in barrels with heating instruments to melt the fat for mixing purposes. Tallow can be of different qualities, and some of the grades are as follows: edible tallow, extra fancy tallow, fancy tallow, bleachable fancy tallow, and prime tallow. The different grades refer to the purity/cleanliness of the tallow, and some of the grades may contain appreciable amounts of lard.

Yellow grease is waste grease from food service operations, and it may contain variable mixtures of vegetable and animal fats. Yellow grease is used as a fat for livestock and pet foods, as an industrial raw material, and as a diluent in higher grade inedible fat products such as bleachable fancy tallow. Several animal-vegetable fat blends also are available for feeding to dairy cattle.

Several other feedstuffs (e.g. hominy, dry distillers grain with solubles, and fish meal) contain a moderate amount of fat. Total fat from all sources in diets should be the focus instead of only supplemental fat from major contributors. Because fish meal contains an appreciable amount of 20 and 22-carbon polyunsaturated FA which are very toxic to ruminal bacteria (Hoover *et al.*, 1989), it should be restricted to a maximum 2 to 3% of dietary DM.

Commercial fats

Several commercial fat preparations are available and most of them are marketed as rumen inert sources. These fats fall into two general categories: calcium salts and processed tallow (either hydrolyzed tallow FA or PHT). The calcium salts are made from palm oil (higher in C16:0), soybean oil (higher in C18:2), or blend of fat sources.

Quality Factors

Rendered or processed fats originate primarily as recovered waste fats and can be highly variable in quality. The most important measures of quality are solidification point (titre), saturation/unsaturation (usually measured by IV), total FA (TFA), free FA (FFA), and moisture, insolubles, and unsaponifiables (MIU). Titre and IV both are estimators of unsaturation. Tallow is a triglyceride and has a TFA content of 90% (10% is glycerol). Obviously, FFA should be 100% TFA. Total FA values less than 90% for triglycerides indicate dilution with non-fat substances such as MIU. In many processed fats, the FFA value indicates the amount of "abuse" to which the fat has been subjected, as heating and presence of water tend to hydrolyze or "split" triglycerides to FFA and glycerol. However, livestock can utilize FFA, and their presence alone is a good indicator of fat quality since water has no energy value and its presence dilutes the value of fat. Furthermore, water promotes splitting of fat, rancidity, and corrosion of storage tanks. Good quality fats should contain no more than 1% moisture.

Feeding Practices

One of the original concepts with feeding fat was to reduce body weight (BW) loss during early lactation. However, research results do not support this concept. The extra energy consumed by feeding fat in early lactation primarily supports higher milk yield. Since the feeding of fat does not appear to reduce BW loss during early lactation and palatability problems sometimes exist with certain fats, it is advised not to feed high levels of fat until 30 days postpartum. This strategy will allow the cow some time to adjust to the lactational phase before fat is included in the diet; after all, the main strategy during the first 2 to 4 weeks of lactation should be to maximize DM intake rather than energy intake. After intake has reached an acceptable level, then energy intake can be the focus.

There is some evidence that feeding fat may improve reproductive efficiency of dairy cows, independent of any effects on energy balance of the cows (Lucy *et al.*, 1991; Staples *et al.*, 1998). Fat supplementation may increase the number and size of ovarian follicles, increase plasma concentration of progesterone, reduce secretion of prostaglandin metabolites, and increase lifespan of the corpus luteum (Staples *et al.*, 1998). The FA profile of the supplemental fat is important for its positive impact on reproduction, with

linoleic acid being one of the causative FA. Therefore, this has lead to increased interest in feeding natural fat sources higher in linoleic acid and the development of calcium salts higher in linoleic acid (Table 7.1).

The need for fat in diets should be based on the animals' milk yield and body condition, quality of forages in diets (poorer quality forages are lower in energy), and the level of DM intake by animals. As a guideline, cows can efficiently utilize as much dietary fat as produced in milk, with appropriate adjustments for BW change (subtract amount lost or add amount gained in adipose tissue) (Palmquist and Eastridge, 1991). The amount of fat that can actually be included in diets depends on the fat source, level of DM intake, and fiber level in the diet. The importance of fat source in this regard was alluded to earlier. Cows consuming higher amounts of DM and consuming diets adequate to high in fiber compared to diets marginal in fiber can handle a higher level of dietary fat. Using the fiber equations by Jenkins (1997) are more applicable for typical diets and should be used with caution with low forage diets based on high NDF from nonforage fiber sources.

Since DM intake influences the amount of fat to include in diets, fat levels to feed should be expressed as a percentage of DM intake rather than on a weight basis. To provide supplemental fat, the natural fats should be used as a first priority because of their lower cost. Generally speaking, oilseeds can be added to provide an additional 2% fat or tallow and animal-vegetable blends can be used to provide 2.5 to 3% supplemental fat to diets (Table 1). The benefit of commercial fats become more apparent when total dietary fat level must exceed 5% of dietary DM. Commercial fats are important for their rumen inertness and are convenient due to their ease of handling. Price, availability, and characteristics that relate to palatability, inertness, and digestibility are important for making comparisons among different commercial fats.

Insoluble soaps formed between fatty acids and cations, especially calcium and magnesium, in the lower portion of the small intestine may reduce the absorption of calcium and magnesium (Jenkins and Palmquist, 1984; NRC, 2001). The evidence for this is equivocal, but magnesium and calcium should be increased 20 to 25% in diets containing supplemental fat.

Effects of Supplemental Fat on Milk Composition

Adding fat to diets for lactating cows generally increases milk yield (if energy is limiting in the diet) and increases milk protein yield but decreases milk protein concentration, typically by 0.1 to 0.2 percentage units. The metabolic processes attributing to this decline in milk protein concentration has reviewed considerable attention, but the mechanism may still be uncertain (Schingoethe, 1996; Wu and Huber, 1994).

Interest in the effects of supplemental fat on the FA composition of milk fat has increased and recently has been extensively reviewed (Jensen, 2002). About 50% of the fat in milk is derived from de novo synthesis in the mammary gland (most of the 4:0 to 14:0 and about 50% of 16:0) and 50% from performed FA from either the diet or adipose tissue; however, the relative contribution by preformed FA is higher during early lactation because of mobilization of adipose tissue. Supplemental fat also increases the contribution of performed FA, thereby increasing the long-chain FA and decreasing the short-chain FA in milk. Supplemental dietary fat may increase or have no impact on milk fat percentage, and feeding fat may decrease milk fat percentage if ruminal fermentation is adversely affected, possibly related to the increased trans-18:1 in milk (Jensen, 2002).

Alteration of the FA composition of milk and the location of specific FA on glycerol can affect the processing properties of milk and the nature (especially firmness) of dairy products (such as cheeses). Much interest continues in the concentration of conjugated linoleic acid (CLA) in milk because of its anti-carcinogenic properties found in laboratory animals. Research continues with attempting to increase CLA in milk by feeding different dietary sources of fat, altering biohydrogenation in the rumen, understanding the role of delta-9 desaturase in the mammary gland, and understanding why cows on pasture have higher CLA in milk than cows fed stored feeds.

Fat Analysis

Analysis of feeds for fat is not customary for many labs. Only high-fat feeds, by-product feeds, or blended feed mixtures containing supplemental fat are worthy of fat analysis. Some by-product feeds are quite variable in fat, and thus, fat analysis would be advised.

Fat in feeds is usually analyzed either by ether extraction (EE) or by gas chromatographic methods. The EE procedure results in higher values because it includes everything that is soluble in ether. The gas chromatographic methods provide a more precise analysis because FA are actually measured. The FA content can be generally estimated from EE values by the following: forage EE x 0.50; concentrate EE x 0.85, and tallow x 0.90. It is important to know which method is used so dietary levels of fat can be consistent and accurate.

Conjugated linoleic acid

Dairy products and meat from ruminant animals are important sources of nutrients, supplying high quality protein, energy, and a variety of minerals and vitamins. Research during the last few years reveals other nutritional benefits to the consumption of ruminant

food products, particularly dairy products. There is one compound in particular, conjugated linoleic acid (CLA), that excites scientists, consumers, and producers, and may have far-reaching, positive effects on milk and meat consumption. CLA brings a promising approach to redesigning food because milk has high levels of CLA, and CLA has been shown to have numerous potential benefits for human health, including potent cancerfighting properties.

CLA Chemistry

All milk contains some fat (3.2 to 4.7%), but within milk there are a couple hundred different types of fat and fatty acids. The major fatty acids in milk fat range from 4 to 20 carbon chain length. Linoleic acid, an essential dietary fatty acid, contains 18 carbons (C) with two double bonds (C18:2) (see Figure 1-C). CLA is a term for specific isomers (forms) of linoleic acid with conjugated double bonds (double bonds adjacent to each other C=C-C=C).

Of the 20 different isomers of CLA that have been identified, the cis 9-trans 11 (9c, 11t) form (commonly called "rumenic acid") is believed to be the most common natural form of CLA. The trans 10-cis 12 isomer has also been identified. Researchers are also identifying and studying other potentially active isomers.

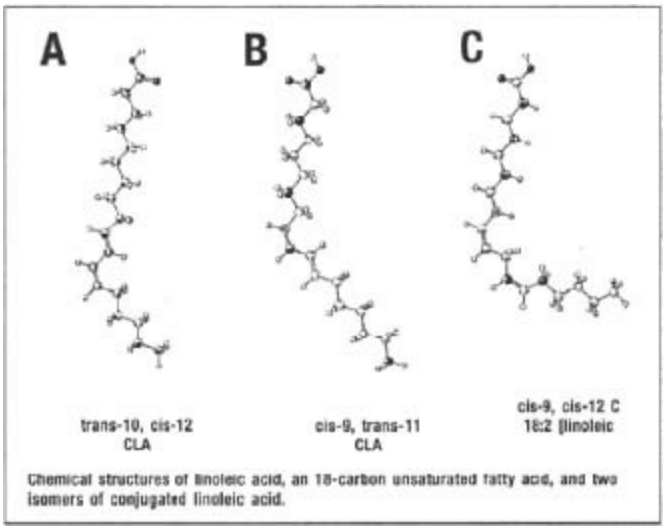


Fig 7.2: Isomers of CLA

CLA was first isolated and identified in the 80's by Dr. Michael Pariza at the University of Wisconsin. Dr. Pariza isolated CLA while looking for a compound in cooked hamburger that prevented skin cancer in mice. The cis 9, trans-11 isomer is the form most commonly found in ruminant animals, and is the isomer thought to be most effective in lowering the risk of cancer in humans.

CLA Synthesis

Ruminal bacteria are key to the formation of CLA which explains why CLA production is unique to and found almost exclusively in food products (milk and meat) produced from ruminant animals. CLA is an intermediate of the biohydrogenation of linoleic acid to stearic acid. Biohydrogenation is a collective term used to describe the conversion of unsaturated to saturated fatty acids via isomerization and hydrogenation of unsaturated fatty acids by rumen bacteria. This process is speculated to be a defense mechanism by rumen bacteria against potentially toxic unsaturated fatty acids. During biohydrogenation of fatty acids, including the CLA intermediate, fatty acids are continually leaving the rumen, being absorbed across the small intestine, and incorporated into milk fat. The biohydrogenation and formation of CLA from linoleic acid has long been known as a source of CLA in the rumen. Another major source of CLA is generated by other intermediates of biohydrogenation and sequential CLA synthesis in the mammary gland. Biohydrogenation of oleic, linoleic, and linolenic acid all result in trans-11 (18:1). The trans-11 can be converted to 9c, 11t CLA in the mammary gland by the tissue delta 9 desaturase enzyme. The importance of both of these sources has been studied, and mammary synthesis may account for up to 70 to 80% of total CLA found in milk. However, it is important to keep in mind that the substrate for mammary CLA synthesis originates in the rumen through microbial biohydrogenation. This demonstrates that the rumen is still a primary source of CLA production.

Boosting concentrations of CLA in milk

Surveys have found an eight-to-ten fold variation in CLA concentrations in milk. This suggests that nutrition and herd management practices may create a rumen environment that alters biohydrogenation of fatty acids and, in turn, the CLA concentrations in milk. In

Table 2 are listed the nutritional and ruminal factors that affect CLA concentration in milk fat.

Increases in CLA in milk were first noticed over 65 years ago when cows were turned onto spring pasture. The interest in CLA has renewed the interest in increasing the CLA content of milk. A study at Penn State reported a two-fold increase in CLA with pasture (5.4 to 10.9 mg/g of fat). This increase has been attributed to increased supply of fat substrate, and to potential changes in the rumen environment and synthesis in the mammary gland. Adding supplements to the ration of grazing cows may diminish this effect.

Replacing conserved forages with fresh pasture clearly increases CLA concentrations in milk. A study comparing confinement feeding of a TMR to pasture + TMR and pasture + concentrate clearly shows that feeding pasture elevated the CLA in milk. CLA in the milk of cows fed a TMR was constant at 6 mg/g fat for the 18 week study. Cows fed pasture plus concentrate had elevated CLA in milk by week 4 and 6, and the concentration peaked at 18 mg/g fat at week 18. Cows fed pasture + TMR had CLA concentrations closer to that of cows fed TMR in confinement.

Fat supplementation and feed sources richer in unsaturated fatty acids have been shown to increase CLA in milk. Unsaturated plant oils increase CLA more than feeding saturated animal fat sources. This is due to the lipid substrate available by the plant oils for biohydrogenation to CLA and CLA precursors in the rumen. It follows then that increasing levels of plant oil and feeding calcium salts of plant oils will increase levels of CLA in milk. Feeding plant seeds high in fat such as soybean, cottonseed, sunflower, and flax (linseed) also increases CLA in milk.

However, if seeds are fed in a raw form, there is little change on CLA in milk. The seed coat must be broken making the fat available to rumen biohydrogenation for CLA production. Supplementing with fish oil has been shown to increase CLA content of milk when dairy cows on pasture were fed high oil seeds, the CLA content of milk increased more than when feeding pasture alone.

If we think back to the biosynthesis of CLA and the nutritional factors that increases CLA in milk, it is obvious there are many pathways and nutritional factors that can increase CLA content of milk. Employing these nutritional factor together may increase CLA content of milk beyond any one factor. A study was conducted to increase the levels of CLA in milk by affecting rumen biohydrogenation and supplying lipid substrate. Cows were fed a TMR with the addition of corn oil, fish oil, or both. Including fish oil has been shown to inhibit biohydrogenation allowing more intermediate products of biohydrogenation, including CLA and CLA precursors, to escape the rumen and be incorporated into milk. Lipid substrates such as corn oil have shown to increase CLA content of milk by providing more unsaturated fatty acids for biohydrogenation. When cows were fed both corn oil and fish oil in combination, CLA content in milk increased ten-fold.

Potential health benefits

The potential benefit of CLA in human health is the major reason for the excitement and interest in CLA. The major interest surrounding CLA is the anti carcinogenic or anti-cancer effects. Much of the research to date has been with laboratory animal models. CLA can reduce new tumor growth and destroy existing tumor cells. CLA has killed existing cancer cells in colon, ovarian and prostate carcinoma, leukemia, melanoma, and breast tumors. CLA enriched butter inhibited rat mammary tumor yield by 53%, clearly showing the cis-9 trans-11 isomer was anticarcinogenic. In addition to the anti-carcinogenic properties, other positive health benefits demonstrated in animal models include:

- Reduced atherosclerosis
- Enhanced immune system
- Prevention and treatment of diabetes
- Weight reduction; reduced body fat and increase body protein
- Enhanced bone formation

As research continues on many fronts, the specific physiological effects and the responses will be better defined.

Importance of dietary CLA in pig feeding

Concerns about the development of antibiotic-resistant bacteria have led to feeding bans on several antibiotics in the European Union. One alternative to antibiotics may be the incorporation of conjugated linoleic acid (**CLA**) into swine diets. Research indicates that CLA can modify the immune system of weanling pigs by altering the type and number of immunocytes (Bassaganya- Riera *et al*., 2001). Pigs fed diets supplemented with CLA have greater gain: feed efficiency and leaner carcasses than pigs not fed CLA (Dugan *et al*., 1997; Eggert *et al*., 1999). A portion of the increase in efficiency and lean growth could perhaps be attributed to CLA's modulation of the immune response in swine (Bassaganya-Riera *et al*., 2001). However, researches on the effect of CLA on the overall performance of pigs are very limited.

Conclusion

Fats are very useful for increasing energy density of diets for high-producing dairy cows. Similar to other feeding changes, fat should be gradually introduced into diets. Physical and chemical properties of available fat sources, animal's milk yield, body condition, and level of DM intake, and associated costs are factors for consideration when feeding fat to dairy cows. Use of feed grade fats on dairy farms is expected to continue because of increasing milk yield per cow. Different commercial or specialty fats will continue to be available for feeding upper levels of fats in diets and for targeted roles based on new research. The presence of a compound (CLA) in ruminant fat with such potent health promoting effects has been an unanticipated discovery. The ability to enhance the concentration of CLA through manipulation of the dairy ration demonstrates the feasibility of producing CLA enriched dairy products. As consumers become more conscious of the link between diet and health, milk designed to have enhanced levels of CLA may provide new market opportunities for milk and milk products such as butter and cheese.

Table 7.1. Fatty acid composition and characteristics of different fat sources

Fat Source	Fatty acids (weight %)							Fat (EE) (%)	C18 Unsat.FA	IV (%)	Fat digestibility (%)
	14:0	16:0	16:1	18:0	18:1	18:2	18:3				
Oil seeds											
Cotton seed	0.08	22.7	0.8	2.3	17.0	51.5	0,2	19.3	68.7	107	86
Linseed oil cake	—	5.3	—	4.1	20.2	12.7	53.3	100	86.2	185	86
Soybeans	0.1	10.3	0.2	3.8	22.8	51.0	6.8	19.0	80.6	131	86
Sunflower	—	5.4	0.2	3.5	45.3	39.8	0.2	41.9	85.3	113	86
Animal fats											
Tallow	3.0	24.5	3.7	19.3	40.9	3.2	0.7	99.8	44.8	48	68
Yellow grease	1.8	22.1	3.5	11.5	43.7	14.6	0.9	99.0	59.2	72	—
Commercial fats											
Calcium salt	1.3	48.6	1.1	4.1	36.5	7.8	0.3	84.5	44.6	49	86
Megalac	—	47.1	—	4.7	36.5	9.4	1.2	85.0	47.1	53.0	—
Rumolac	—	9.0	—	9.0	31.5	38.5	—	82.9	70.0	98.0	—

Table 7.2. Factors affecting CLA Concentrations in Milk Fat

Factors	Effect on CLA concentration in milk fat
Forages	
Pasture versus TMR[1]	Increased with consumption of pasture
Forage: concentrate ratio[2]	Increased with high forage diet
Maturity of forage[1]	Increased with less mature forage
Plant oils	
Unsaturated versus saturated[3]	Increased with the addition of unsaturated oils
	Increased by feeding higher levels

Amount of plant oils[3]	
Calcium salts of plant oils[3]	Increased with increasing amounts
Plant seeds	
Raw seeds	No Effect
Processed seeds[3]	Increased over raw seeds
Other	
Plant oil versus animal fats[3]	Increased with plant oils
Fish oil[2]	Increased in relation to level fed in the diet

[1]CLA increases may be due to both addition of lipid substrate and modified rumen environment

[2]CLA increases may be due to a modified rumen environment

[3]CLA increases may be due to addition of a lipid substrate

References

Bassaganya-Riera, J., Hontecillas-Magarzo, R., Bregendahl, K., Wannemuehler, M. J. and Zimmerman. D.R. 2001. Effects of dietary conjugated linoleic acid in nursery pigs of dirty and clean environments on growth, empty body composition, and immune competence. *Journal of Animal Science*, **79**:714–721.

Bauman, D.E. and Currie, W.B. 1980. Partitioning of nutrients during pregnancy and lactation; a review of mechanisms involving homeostasis and homeorhesis. *Journal of Dairy Science*, **63:** 1514-1529.

Chen, Shi Yong, Guo Yu Ming, Xia ZhaoGang and Yuan Jian Min. 2003. Effects of different types of polyunsaturated fatty acids on humaoral immune function and hepatic lipid peroxidation of laying hens. *Acta Nutrimenta Sinica*, **25**: 383-388.

Cook, M.E. 1991. Nutrition and immune response to the domestic fowl. *Critical Reviews in Poultry Biology*, **3:** 167-189.

Coppock, C.E., Lanham, J.K. and Horner, J.L. 1987. A review of the nutritive value and utilization of whole cottonseed, cottonseed meal and associated by-products by dairy cattle. *Animal Feed Science and Technology*, **18**: 89-129.

Dugan, M. E. R., Aalhus, J. L., Schaefer, A. L. and Kramer, J. K. G. 1997. The effect of conjugated linoleic acid on fat to lean partitioning and feed conversion in pigs. *Canadian Journal of Animal Science*, **77**:723–725.

Eggert, J. M., Carroll, A. L., Richert, B. T., Gerrard, D. E., Forrest, J. C., Bowker, B. C., Wynveen, E. J., Hammelman, J. E. and Schinckel, A. P. 1999. Effects of conjugated linoleic acid (CLA) on the growth, carcass composition and pork quality of two genotypes of lean gilts. *Journal of Animal Science*, **77**(Suppl. 1):178 (Abstr.).

Faldet, M.A. and Satter, L.D. 1991. Feeding heat-treated full fat soybeans to cows in early lactation. *Journal of Dairy Science*, **74**: 3047-3054.

Firkins, J.L. and Eastridge, M.L. 1994. Assessment of the effects of iodine value on fatty acid digestibility, feed intake, and milk production. *Journal of Dairy Science*, **77**: 2357-2366.

Firkins, J.L., Eastridge, M.L., St-Pierre, N.R. and Noftsger, S.M. 2001. Effects of grain variability and processing on starch utilization by lactating dairy cattle. *Journal of Animal Science*, **79** (E. Suppl.):E218-E238.

Friedman, A. and Sklan, D. 1997. Effect of dietary fatty acids on humoral immune response of turkeys. *British Poultry Science*, **38:** 342-348.

Fritsche K.L. and Cassity, N.A. 1992. Dietary n-3 fatty acids reduce antibody-dependent cell cytoxicity and alter eicosanoid release by chicken immune cells. *Poultry Science*, **71**: 1646-1651.

Fritsche, K.L., Cassity, N.A. and Huang, S. 1991. Effect of dietary fat source on antibody production and lymphocyte proliferation in chickens. *Poultry Science*, **70**: 611-617.

Hamdy, A.M.M., Soliman, M.A.H., Abdalla, A.G.M. and Ismail, Z.S.H. 2003. Effects of dietary polyunsaturated fatty acids on some immune responses of broiler chicks. *Egyptian Poultry Science Journal*, **23**: 601-616.

Hoover, W.H., Miller, T.K., Stokes, S.R. and Thayne, W.V. 1989. Effects of fish meals on rumen bacterial fermentation in continuous culture. *Journal of Dairy Science*, **72**:2991-2998.

Hsu, J.T. and Satter, J.D. 1995. Procedures for measuring the quality of heat-treated soybeans. J*ournal of Dairy Science*, **78**:1353-1361.

Humphrey, B.D., Koutsos, E.A. and Klasing, K.C. 2002. Requirements and priorities of the immune system for nutrients. 69-77 in :

Nutritional Biotechnology in the feed and food industries : Proceeedings of Alltech's 18th Annual Symposium. T.P. Lyons and K.A. Jasques, eds. Nottingham, University Press, Nottingham, UK.

Jenkins, T. 1997. Success of fat in dairy rations depends on the amount. *Feedstuffs*, **13:**11-12.

Jenkins, T.C. and Palmquist, D.L. 1984. Effect of fatty acids or calcium soaps on rumen and total nutrient digestibility of dairy rations. *Journal of Dairy Science*, **67**: 978-986.

Jensen, R.G. 2002. The composition of bovine milk lipids: January 1995 to December 2000. *Journal of Dairy Science*, **85:**295-350.

Korver, D.R. and Klasing, K.C. 1997. Dietary fish oil alters specific and inflammatory immune responses in chicks. *Journal of Nutrition*, **127**: 2039-2046.

Korver, D.R. and Klasing, K.C. 1995. n-3 polyunsaturated fatty acids improve growth rate of broiler chickens and decrease interleukin-1 production. *Poultry Science*, **74**: (Suppl.1) 15. (Abstr.)

Koutsos, E.A. and Klasing, K.C. 2001. Interaction's between the immune system, nutrition and productivity of animals. pages 173-190 in: Recent advances in Animal Nutrition, 2001, P.C. Garnsworthy and J. Wiseman, eds. Nottingham University Press, Nottingham.

Liu, D., Veit, H.P., Wilson, J.K. and Denbow, D.M. (2003). Material dietary lipids alter bone chemical composition, mechanical properties and histological characteristic of progeny of japanese quails. *Poultry Science*, **82:** 43-55.

Lucy, M.D., Staples, C.R., Michel, F.M., Thatcher, W.W. and Bolt, D.J. 1991. Effect of feeding calcium soaps to early postpartum dairy cows on plasma prostaglandin F2a, luteinizing hormone, and follicular growth. *Journal of Dairy Science*, **74**:483-489.

National Research Council. 2001. Nutrient requirements of dairy cattle. 7th rev. ed. Natl. Acad. Sci., Washington, DC.

Palmquist, D.L. and Conrad. H.R. 1971. High levels of raw soybeans for dairy cows. *Journal of Animal Science*, **33:** 295. (Abstr.)

Palmquist, D.L. and Eastridge, M.L. 1991. Dietary fat effects on milk yield and composition. California Animal Nutrition Conference, pp. 2-25.

Parmentier, H.K., Awati, A., Nieuwland, M.G.B., Schrama, J.W. and Sijben, J.W.C. 2002. Different sources of dietary n-6 polyunsaturated fatty acids and their effects on antibody responses in chickens. *British Poultry Science*, **43**: 533-544.

Reddy, P.V., Morrill, J.L. and Nagaraja, T.G. 1994. Release of free fatty acids from raw and processed soybeans and subsequent effects on fiber digestibilities. *Journal of Dairy Science*, **77**: 3410-3416.

Schingoethe, D.J. 1996. Dietary influence on protein level in milk and milk yield in dairy cows. Animal Feed Science and Technology, **60**:181-190.

Selvaraj, R.K. and Cherian, G. 2004. Dietary n-3 fatty acids reduce the delayed hypersensitivity reaction and antibody production more than n-6 fatty acids in broiler birds. *European Journal of Lipid Science and Technology*, **106**: 3-10.

Sijben, J.W.C. 2002. Modulation of the chicken immune cell function by dietary polyunsaturated fatty acids. *Poultry Science*, **81**: 155.

Staples, C.R., Burke, J.M. and Thatcher, W.W. 1998. Influence of supplemental fats on reproductive tissues and performance of lactating cows. *Journal of Dairy Science*, **81**: 856-871.

Tice, E.M., Eastridge, M.L. and Firkins, J.L. 1993. Raw soybeans and roasted soybeans of different particle sizes. 1. Digestibility and utilization by lactating cows. *Journal of Dairy Science*, **76**: 224-235.

Torki, M., Arshami, J., Shahroodi, F.E., Afshar, J.T. and Golian, A. 2003. Effects of dietary n-3 and n-6 polyunsaturated fatty acids on performance and humoral immune response in broiler chicks. *Iranian Journal of Agricultural Science*, **34**: 115-125.

Vilchez, C., Touchburn, S.P., Chavez, E.R. and Chan, C.W. 1992 Effect of feeding palmitic, oleic, and linoleic acids to Japanese quail hens (*Coturnix coturnix japonica*). 2. Maternal diets and stage of incubation on the lipid metabolism of quail embryos. *Poultry Science*, **71**:1032-1042.

Wang, Y.W., Cherian, G., Sunwoo, H.H. and Sim, J.S. 2000. Dietary polyunsaturated fatty acids significantly affect laying hen lymphocyte proliferation and immunoglobulin G concentration in serum and egg yolk. *Canadian Journal of Animal Science*, **80**: 597-604.

Wu, Z. and Huber, J.T. 1994. Relationship between dietary fat supplementation and milk protein concentration in lactating cows: a review. *Livestock Production Science*, **39**:141-155.

Xia, Z.G., Guo, Y.M., Chen, S.Y. and Yuang, J.M. 2003. Effects of dietary polyunsaturated fattya cids on antibody production and lymphocyte proliferation of laying hens. *Asian Australasian Journal of Animal Science*, **16**: 1320-1325.

Zaki, M.M. and Hady M.M. 1995. Impact of different dietary fat sources on performance and immune response of broiler chickens. *Veterinary Medical Journal of Giza*, **43**: 183-192.

8 Phytobiotics

Phytobiotics are plant derivatives such as herbs, plant extracts or spices and have a wide range of activities such as stimulation of feed intake, growth and endogenous secretions in the gut. They act as immunomodulators resulting in decreased mortality and also have coccidiostatic, anti microbial, anthelminthic and anti-inflammatory activities. Phytobiotics also possess hepatoprotective and hepatogenic properties, which tone up liver resulting in increased nutrient utilization and better performance. Herbs like *Achyranthes aspera* (Prickly Chaff Flower, Devil's Horsewhip, Apamarga), *Andrographis paniculata* (Green chirayta, King of bitters, Kalamegha), *Azadirachta indica* (Neem), *Boerhaavia diffusa* (Spreading Hogweed, Punarnava), *Eclipta alba* (False Daisy, Bhringaraj), *Ichnocarpus frutescens* (Black creeper, Utpalagopa), *Terminalia chebula* (Black myrobalan, Haritaki). These herbs have hepato-stimulant, hepato-protective, immunomodulatory and antioxidant activities (Sadekar *et al*., 1998; Manu and Kuttan, 2009; Michels *et al*., 2011, Dash *et al*., 2007). Further, they optimize digestion and metabolism resulting in better protein utilization, improved mucosal function and reduced cost of metabolic deamination. Andrographolide and 14-deoxy-11, 12-didehydro-andrographolide isolated from *Andrographis paniculata* inhibits free radical activities and lipid peroxidation. Inhibition of lipid peroxidation in meat prevents free radical production thereby preserving meat composition, colour and improvement in shelf life. In addition, it has been studied that a herb *Terminalia chebula* helps to reduce stress (Selvakumar *et al*., 2007).

Use of herbs for treatment of animals and human beings has been from very ancient times. Herbal medicine has its origins in ancient cultures including those of the Egyptians, American, Indians and Chinese. Varieties of plants of medical importance were used by man even during the Treta Yuga. The earliest compilation of drugs and illness appeared in "AYURVEDA" which is claimed to have been written around 3000 BC. However, the concept gained momentum in the scientific world only in the later part of last century. Various herbs or herbal products are used for treatment of different diseases. It has been estimated that around 8000 different types of natural medicines are in use in China. Most of the natural medicines are made of different plant products.

The herbs and their extracts increase appetite, stimulate digestive enzymes, modulate immune system and have anti-helminthic and anti-coccidial effect. In recent years, several studies have been carried out to test the efficacy of different herbs and their secondary metabolites on the growth, immunocompetence and production traits of livestock and poultry. However, it is beyond the scope of this chapter to discuss all of them. Hence, an attempt has been made to discuss the role of some commonly used herbs and plant secondary metabolites in livestock and poultry.

Curcuma longa (Haldi)

Turmeric has a long tradition of use in the Chinese and Ayurvedic systems of medicine, particularly as an anti-inflammatory agent, and for the treatment of flatulence, jaundice, menstrual difficulties, hematuria, hemorrhage and colic. Turmeric is known as stomachic, blood purifier, and is useful in many conditions like common cold, leprosy intermittent fever, infections of liver, dropsy, purulent ophthalmia, otorrhea, pyogenic infections, wound healing and infections etc.

Turmeric can also be applied topically to relieve pain and inflammation. Current research has focused on turmeric's antioxidant, hepatoprotective, anti-inflammatory, anticarcinogenic, and antimicrobial properties, in addition to its use in cardiovascular disease and gastrointestinal disorders. Some of the studies on turmeric by various workers have been mentioned in this section.

Soni *et al*. (1992) studied the effect of extracts of turmeric (*Curcuma longa*), Garlic (*Allium sativum*) and Asafetida (*Ferula asafetida)* on aflatoxin production by *Aspergillus parasiticus in vitro* and found 90% inhibition of aflatoxin at concentration of 5-10mg/ml. They also reported the reverse changes in damaged liver, fatty change, necrosis and bilary hyperplasia by these feed additives. Kurkure *et al*. (2000) concluded that turmeric treatment can only partially ameliorate low grade aflatoxicosis in chicks. Sultan (2003) reported that turmeric (feed additive) at a level of 0.5% enhances overall performance of broiler chickens.

Emadi and Kermanshahi (2006) observed that addition of turmeric rhizome powder (TRP) into the diets significantly decreased

relative abdominal fat pad and heart weights to live body weight. So they concluded that TRP may improve carcass quality and produce leaner meat. Further, they pointed out that use of TRP as a reducing heart weight factor, may show some improvement in circulatory and respiratory systems.

Terminalia arjuna

Arjuna tree is found throughout the sub-Himalayan tracts, the Deccan region, Srilanka and the south eastern countries. The bark is used to heal wounds. It is also used in heart-disease, contusions, and fractures. Juice of leaves is used in ear-aches. It is one of the best herbs for heart disease (prevents and helps in the recovery of) angina and heals heart tissue scars after surgery. Also useful for bile, edema, fractures, broken bones, diarrhea malabsorption and venereal disease as well as external treatment for ulcers, acne, skin disorders. Arjuna bark is used in the form of decoction (1 in 10) in doses of half to one ounce in hemorrhages. Also, it is used in diarrhea, dysentery and sprue. It is also useful in bilious affections, and as an antidote to poisons. It is used as a remedy for scorpion sting.

In recent years, lot of studies has been carried out to study the medicinal properties of different parts of *T. Arjuna*. Some of these studies have been briefly discussed.

Khanna *et al*. (1996) observed that supplementation of *Terminalia arjuna* bark powder (100 mg/kg diet) to hyperlipaemic rats lowered serum lipids and increased HDL cholesterol levels. Sivalokanathan *et al*. (2006) studied on ethanolic extract of arjuna bark on carbohydrate metabolizing enzymes of N-nitrosodiethylamine induced hepatocellular carcinoma in Wistar albino rats. The plasma and liver glycolytic enzymes such as hexokinase, phosphoglucoisomerase, aldolase were significantly increased in cancer induced animals while glyconeogenic enzyme; glucose-6-phosphatase was decreased. These enzymes were reverted significantly to near normal range in treated animals after oral administration of *T. arjuna* for 28 days. The modulation of the enzymes causing depletion of energy metabolism leads to inhibition of cancer growth. This inhibitory activity may be due to the anticancer activity of constituents present in the ethanolic extract of *T. arjuna*.

Raghavan and Kumari (2006) reported that ethanolic extract (250 and 500 mg/kg body weight) of Terminalia arjuna stem bark in alloxan induced diabetic rats produced significant reduction in lipid peroxidation. The effect of oral *T. arjuna* at the dose of 500 mg/kg body weight was more than the 250 mg/kg body weight. The extract also causes a significant increase in superoxide dismutase, catalase, glutathione peroxidase, glutathione-s-transferase glutathione reductase and glucose-6-phosphate dehydrogenase, reduced glutathione, vitamin A, vitamin C, vitamin E, total sulfhydryl groups and non protein sulfhydryl groups in liver and kidney of alloxan induced diabetic rats, which clearly shows, the antioxidant property of *T. arjuna* bark. The result indicates that the extract exhibits the antioxidant activity through correction of oxidative stress and validates the traditional use of this plant in diabetic animals.

Emblica officinalis (Amla)

The fruits of *Emblica officinalis* are sour, astringent, bitter, acrid, sweet, cooling, anodyne, ophthalmic, carminative, digestive, stomachic, laxative, aphrodisiac, rejuvenative, diuretic, antipyretic and tonic. They are useful in vitiated conditions of diabetes, cough, asthma, bronchitis, cephalalgia, ophthalmopathy, dyspepsia, colic, flatulence, hyperacidity, peptic ulcer, erysipelas, skin diseases, leprosy, haematogenesis, inflammations, anemia, emaciation, hepatopathy, jaundice, diarrhoea, dysentery, hemorrhages, leucorrhoea, menorrhagia, cardiac disorders, intermittent fevers and greyness of hair. Some of the studies conducted on *E. officinalis* have been mentioned below.

Jain and Khurdiya (2004) reported Indian gooseberry juice contains the highest vitamin C (478.56 mg/100 ml). Bhattacharya *et al*. (1999) reported that the antioxidant activity of *E. officinalis* may reside in the tannoids of the fruits of the plant, which have vitamin C like properties, rather than vitamin C itself. Rege *et al*. (1999) reported *Emblica officinalis* strengthened the defence mechanisms against free radical damage induced during stress. The effect of *Emblica officinalis* appeared to depend on the ability of target tissues to synthesize prostaglandins.

Bhattacharya *et al*. (2000) concluded that anti-stress activity of *E. officinalis* may be at least partly due to its tendency to normalize stress-induced perturbations in oxidative free radical scavenging activity as several stress-induced diseases, including the process of aging, may be related to accumulation of oxidative free radicals in different tissues. Nosal'ova *et al*. (2003) reported that antitussive activity of the dry extract of *Emblica officinalis* is not only due to antispasmolytic and antioxidant efficacy effects but also due to its effect on mucus secretion in the airways. Panda and Kar (2003) reported that the ethanolic extract from the fruits of *Emblica officinalis* may potentially ameliorate the hyperthyroidism with an additional hepatoprotective benefit.

Rajak *et al*. (2004) reported chronic *Emblica officinalis* administration causes myocardial adaptation by augmenting endogenous antioxidants and protects rat hearts from oxidative stress associated with ischemic-reperfusion injury. Rao *et al*. (2005) reported the oral administration of amla extracts to the diabetic rats slightly improved body weight gain and also significantly alleviated various oxidative stress indices of the serum of the diabetic rats. In addition, the decreased albumin levels in the diabetic rats were significantly improved with amla. These results form the scientific basis supporting the efficacy of amla for relieving the oxidative

stress and improving glucose metabolism in diabetes. Sancheti *et al*. (2005) reported *Emblica officinalis* (Family: Euphorbiaceae) indigenous to India, is valued for its unique tannins and flavonoids, which contain very powerful antioxidant properties. Scartezzini *et al*. (2006) reported *Emblica* fruit contains ascorbic acid (0.4%, w/w), and that the ayurvedic method of processing increases the healthy characteristics of the fruit due to a higher antioxidant activity and a higher content of ascorbic acid (1.28%, w/w). It has also been found that Vitamin C accounts for approximately 45-70% of the antioxidant activity.

Azadirachta indica (Neem)

Neem is very effective against any kind of infection occurring in body. It is also regarded as herbal antibiotic. It helps in healing of wounds, keeps wounds unaffected by infections, reduces inflammation and reduces pus formation. It also helps in preventing itching. It is helpful in worm infestation. It is also helpful in relieving from burning sensation in acidity. It helps in purifying blood and is useful in diabetes and urine related problems. It also helps in relieving from respiratory problems as it is mucolytic and expels out extra mucus present in the respiratory tract. It is very effective in skin related ailments, it also promotes digestion. It is very effective in fever especially caused in malaria. It improves eyesight. It removes foul odor from mouth and strengthens gums and teeth.

The bark is bitter, astringent, acrid, refrigerant, depurative, antiperiodic, vulnerary, demulcent, insecticidal, liver tonic, expectorant, urinary astringent, anthelmintic, pectoral and tonic. It is useful in vitiated conditions of pitta, hyperdipsia, leprosy, skin diseases, eczema, leucoderma, pruritus, intermittent and malarial fevers, wounds, ulcers, burning sensation, tumour, tubercular glands, anorexia, vomiting, dyspepsia, intestinal worms, hepatopathy, cough, bronchitis, urorrhea, diabetes, inflammation, amenorrhea, lumbago, haemorrhoids, otalgia, syphilis and fatigue. The leaves are bitter, astringent, acrid, depurative, antiseptic, ophthalmic, anthelmintic, alexeteric, appetiser, insecticidal, demulcent and refrigerant. They are useful in vitiated conditions of pitta, burning sensation, leprosy, skin diseases, leucoderma, pruritus, ophthalmopathy, intestinal worms, dyspepsia, ulcers, tuberculosis, boils, eczema and malarial and intermittent fevers. Some of the studies done on poultry is mentioned below.

Uko and Kamalu (2005) observed when raw and autoclaved neem seed kernels were ground and incorporated into standard basal diet at 150 and 225g/kg as substitutes for ground nut cake, there was lymphocytosis and higher levels of neem seed kernels decreased alkaline phosphatase activity in cockerel chicks. In another experiment, Uko and Kamalu (2006) observed when full fat neem seed kernel was incorporated in the diet of cockerel chicks, eviscerated carcass weight decreased and yield increased significantly in cockerel chicks with increased levels of neem kernel. Uko *et al*. (2006) observed when raw full fat neem seed kernel replaced peanut at graded doses in the diet of cockerel chicks, aspartate aminotransferase activity was elevated while cholesterol concentration was lowered. However, activity of alanine aminotransferase and concentrations of bilirubin, creatinine and uric acid did not differ significantly among the cockerels. Further, they observed emaciation and paleness of carcasses, kidney congestion and enteritis.

Lather *et al*. (2002) observed that feeding of neem seed cake to broiler chickens resulted in lower alanine aminotransferase and higher aspartate amino transferase values. However they observed no significant changes in the concentration of uric acid, creatinine, sodium and potassium with feeding of neem seed cake to broiler chickens.

Ocimum sanctum (Tulsi)

Tulsi (*Ocimum sanctum*) contains eugenol, carvacrol, methylchavicol. Tulsi contains a volatile oil consisting of about 70% eugenols as well as methyl eugenol and caryophyllene. Other constituents with likely pharmacological activity include the triterpenoidursonic acid, rosmarinic acid, alkalids, saponins, flavonoids (including apigenin and luteolin and glycosides thereof), phenylpropane glucosides and tannins. The seeds of tulsi contain affixed oil containing five fatty acids, including about 17% linolenic acid and just over 50% linoleic acid.

Tulsi has a long history of medicinal use, and is mentioned in ancient Ayurvedic text such as Charak Samhita. Tulsi is used for treatment of variety of conditions including pain, fever, vomiting, bronchitis, earache and diseases of heart and blood. It is also used in case of diabetes, arthritis and asthma. Fresh leaves taken with black pepper used as a prophylactic against malaria and a decoction of the root are recommended for malarial fever. The leaf juice is used in chronic fever, hemorrhage, dysentery and dyspepsia. It is used also as an anthelmentic and is topically used for ringworm and skin diseases. Its pounded leaves are mixed with sandalwood and applied topically for relief in headache. Tulsi seeds are used in anti leucoderma preparations. Zheljazkov *et al*. reported that essential oil content of *O.bacilium* had *in vitro* activity against *Leishmania donovani*. Some of the studies in tulsi pertaining to poultry have been mentioned below.

Gupta and Charan (2007) studied the non toxic doses of *Ocimum sanctum* in broiler chicken. It was found that maximum weight gain was observed in the group of chickens treated with 200 mg of dried leaves powder per birds daily for 15 days as compared to control group. Hindustani and Singh (2006) reported that Zeetress, a herbal preparation containing extract of *Ocimum sanctum* and *Withania somnifera* as major ingredient added to the drinking water @ 5mg per bird from day 3 to 28 and 10mg/bird from day 29 to 42 had immunopotentiating effect in vaccinated chicken with better immune response to Newcastle disease virus vaccine, lower

lesion scores, improvement in live weight gain and feed conversion efficiency. Rao *et al*. (1999) studied the effect of Zeetress, a polyherbal preparation containing *Withania somnifera*, *Ocimum sanctum*, *E. officinalis* on chicken and found that administration of zeetress minimized stress and had a positive effect on weight gain, feed conversion, general resistance and livability. Babu *et al*. (2002) studied the effect of zeetress, a poly herbal compound containing (*Ocimum sanctum*, *Withania somnifera* and *Emblica officinalis*) against cypermethrin induced immunosuppression and reported that cypermethrin significantly reduced the phagocytic, opsonic indices and body weight, where as zeetress significantly restored the affected phagocytic , opsonic indices and body weight.

Batra *et al*. (2004) studied the clinicopathological and immunological effect of *Ocimum sanctum* in chicken. Tulsi leaf powder supplemented @ 5 gm/kg feed did not cause any pathological lesion in the tissue. Delayed type hypersensitivity response against di nitro chloro benzene antigen was significantly increased in the *Ocimum sanctum* leaf powder group, indicating the immuno modulatory activity of tulsi leaf. Lanjewar *et al*. (2008) reported that supplementation of tulsi leaf powder @ 0.5% and 1.0% significantly increased the final body weight and weekly body weight gain in broiler chicken. Feed conversion ratio also improved in 1.0% tulsi leaf powder group. They also noted that serum HDL cholesterol level was significantly increased with supplementation of tulsi in diet. The net profit found was more in birds supplemented with 0.5% Tulsi leaf powder than 1.0% Tulsi leaf powder. They concluded that supplementation of tulsi leaf powder @ 1.0% in broiler diet for 42 days reduced the meat and serum cholesterol with increase in serum HDL–cholesterol. Mamta and Mishra (2006) reported that administration of tulsi dry leaf powder @ 5 g/kg of feed enhanced cell mediated immune response as observed by DTH in terms of increased foot pad thickness to sonicated bacterial cell protein (SBCP) antigen. It also enhanced humoral immune response against *S. pullorum* plain antigen as detected by standard tube agglutination (SAT) test. The present findings indicate that tulsi dry leaf powder has an immunopotentiating effect.

Plant secondary metabolites

Reduced social acceptance of antibiotics in animal feeds leads the search for alternatives, and plants extracts are attractive with consumer opinion that most things ‘natural’ are good. Plants produce an extensive variety of organic compounds derived from their secondary metabolism that are classified in three main groups: saponins, tannins, and essential oils.

Saponins

Effects on animal growth and feed intake

Animal nutritionists have generally considered saponins to be deleterious compounds. In ruminants and other domestic animals the dietary saponins have significant effects on all phases of metabolism, from the ingestion of feed to the excretion of wastes (Cheeke, 1996). Lucerne and soybeans are the main examples of saponin-rich plants that serve extensively in human, ruminant and poultry diets. Recently, a number of studies have reported both beneficial and adverse effects of these compounds in a variety of animals (Sen *et al*., 1998).

Y. schidigera plant extract (saponin containing plant) has been found to improve growth, feed efficiency and health in ruminants (Mader & Brumm, 1987). Quillaja saponins increased the efficiency of in vitro rumen-microbial protein synthesis and decreased degradability of feed protein (Makkar and Becker, 1996). Partially hydrolysed lucerne saponins administered intra-ruminally resulted in a significant reduction in the total protozoa count in the rumen of sheep (Lu and Jorgensen, 1987) which may be the reason for the decrease in feed protein degradability. Saponins are considered to have detrimental effects on protozoa through their binding with sterols present on the protozoal surface. Sterols are absent on bacterial membranes. Yucca extract can also bind NH_4 when ruminal NH_4 concentrations are high, and release it again when ruminal NH_4 is low, providing a continuous and adequate supply of NH_4 for microbial protein synthesis (Hussain & Cheeke, 1995). Supplementation of feed with leaves of Sesbania sesban, known for its high saponin content, has been found to have the potential to improve protein flow from the rumen by suppressing protozoal action there (Newbold *et al*., 1997) but rumen bacteria were observed to be capable of metabolising the antiprotozoal factor. The positive effects of saponins were more pronounced when they were directly administered into the rumen rather than added to the feed (Odenyo *et al*., 1997). Killeen *et al*. (1998) proposed that a surfactant or flocculent action of saponins on the feed constituents that alters the rate of digestion would account for the substrate-dependent nature of the effect of *Y. schidigera* on rumen DM and N digestibility.

Effects on the immune system

Saponin-based adjuvants have the unique ability to stimulate the cell-mediated immune system, as well as to enhance antibody production, and have the advantage that only a low dose is needed for adjuvant activity (Oda *et al*., 2000). There have been quite a few reviews on the immunostimulatory activity of saponins (Barr *et al*., 1998; Sjolander *et al*., 1998). Research thus far has concentrated on Quillaja saponins and derivatives such as immune-stimulating complex vaccines (immunestimulating complexes formed by the combination of cholesterol, saponin, phospholipid and amphiphatic proteins). Quillaja and other saponins either as crude mixtures or as purified compounds have been reported to increase immune-cell proliferation in vitro (Plohmann *et al*., 1997;

Lacaille-Dubois *et al*., 1999). Purified Quillaja saponins boosted antibody production without producing any reaginic antibodies (So *et al*., 1997). Immune-stimulating complexes formulated with Quillaja saponin preparations induced specific cytotoxic T-lymphocyte responses (Coulter *et al*., 1998) and have been reported to induce antibody responses and/or protective immunity in guinea-pig, turkey, cat, rabbit, dog, seal, sheep, pig, cow, horse and monkeys (Mowat *et al*., 1999). The adjuvant action of saponins was, however, not so pronounced in some of the non-mammalian species tested.

The mechanisms of immune-stimulating action of saponins have not been clearly understood, but many explanations have been put forward. Saponins reportedly induced production of cytokines such as interleukins and interferons that might mediate their immunostimulant effects (Jie *et al*., 1984). It is likely that they interact with antigen-presenting cells to induce many of these responses (Barr *et al*., 1998). The incorporation of the saponins into cell or endosomal membranes might expose the incorporated antigen to cytosolic proteases. As against the stimulatory effects on specific immunity components, saponins have also been shown to be able to prevent some non-specific immune reactions such as inflammation (Haridas *et al*., 2001) and monocyte proliferation (Delmas *et al*. 2000; Yui *et al*. 2001). One downstream effector substance whose activity has shown to be lowered by saponin is phospholipase A2 (de Oliveira *et al*., 2001) that causes a decrease in hydrolysis in membrane phospholipids and thereby decreased membrane fluidity. The varied action of saponins might indicate that the immunostimulatory effects of saponins are the result of specific targeting of physiological intermediaries rather than the result of a non-specific effect on cell membrane permeability.

Antioxidant effects

The importance of the antioxidants contained in foods is well appreciated for both preserving the foods themselves and supplying essential antioxidants *in vivo*. However, the term 'antioxidant' is very loosely used. Often, the term is used to describe chain-breaking inhibitors of lipid peroxidation as free radicals generated *in vivo* damage many targets other than lipids, including proteins, DNA and small molecules. These oxidation reactions might lead to an array of adverse biological effects. Some of the protection mechanisms afforded by saponins against this have already been discussed earlier. Zilversmit (1979) hypothesised that atherogenesis might result from phenomena that occur immediately after eating, and that it might be affected by chylomicron remnants. Several researchers (Staprans *et al*., 1994; Wolff and Nourooz-Zadeh, 1996; Ursini *et al*., 1998) expanded Zilversmit's hypothesis and suggested that dietary lipid hydroperoxides, which may be partly generated during digestion in the alkaline pH of the intestine, are the source of chylomicron remnants of lipid hydroperoxides, which are elevated in the postprandial state. These findings emphasise the importance of natural antioxidants in food, and throughout the digestive tract. Usually, polyphenols and carotenoid pigments, being the major nutritional antioxidants in food, attract most of the research in this area. Some saponins have also been found to have antioxidative or reductive activity.

Saponin mixtures present in plants and plant products possess diverse biological effects when present in the animal body. With the information presently available, it is difficult to establish clear functionality and structure– activity relationships regarding the effects of saponins in biological systems. This is largely due to the occurrence of a vast number of saponins with similar chemical structures, and to the complexity of cellular physiological reactions, which are often differently influenced by small and subtle differences in stereo-structures of effector ligands. Other factors that could have substantial influence on saponin actions could be their interactions with other dietary constituents.

Tannins

Definition and classification

The tannins are a group of plant secondary compounds which have been known and used by man for centuries. Their name comes from the French *tan* meaning the bark of the holm oak and other trees used in tanning. From a chemical point of view it is difficult to define tannins since the term encompasses some very diverse oligomers and polymers (Harborne, 1999; Schofield *et al*., 2001). It might be said that the tannins are a heterogeneous group of high molecular weight phenolic compounds with the capacity to form reversible and irreversible complexes with proteins (mainly), polysaccharides (cellulose, hemicellulose, pectin, etc.), alkaloids, nucleic acids and minerals, etc. (Giner-Chavez, 1996; Schofield *et al*., 2001). The tannins have traditionally been divided into two groups: the condensed and the hydrolysable tannins. Hydrolysable tannins (HT) are made up of a carbohydrate core whose hydroxyl groups are esterified with phenolic acids (mainly gallic and hexahydroxydiphenic acid). The condensed tannins (CT), or proanthocyanidins, are non-branched polymers of flavonoids units (flavan-3-ol, flavan-3,4-diol), and usually have a higher molecular weight than the HT (1000-20000 Da compared to 500-3000 Da) (McLeod, 1974; Mueller- Harvey and McAllan, 1992; Mueller-Harvey, 1999). Although this division of the tannins is the most widely accepted, many authors believe it does not fully reflect their chemical complexity (Mueller-Harvey and McAllan, 1992; Mueller-Harvey, 1999).

The distribution of tannins in nature

The tannins are widely distributed throughout the plant kingdom, especially among trees, shrubs and herbaceous leguminous plants (McLeod, 1974). The range of species over which these compounds are found has grown as detection techniques have improved. Despite the general idea that tannins are only found in plant species from tropical or arid/semi-arid areas (Giner-Chavez, 1996), they are found in those of other regions. In general, tannins are more abundant in the parts of the plant that are most valuable to it, e.g., new leaves and flowers (which are more likely to be eaten by herbivores) (Terril *et al*., 1992; Van Soest, 1994). Numerous reports illustrate the effects of environmental and seasonal factors as well as of phenological development. Very briefly, high temperatures, water stress, extreme light intensities and poor soil quality increase the tannin content of plants (Van Soest, 1994).

Effect of tannins in ruminant nutrition

Tannins can be beneficial or detrimental to ruminants, depending on which (and how much) is consumed, the compound's structure and molecular weight, and on the physiology of the consuming species (Hagerman and Butler, 1991). It is important to remember that all the quantities mentioned in this revision should be taken with great caution since different analytical methods and especially different standards (e.g., quebracho, tannic acid, catequin, cyanidin, delphinidin or internal standards from the plant itself etc.) can provide very different —and therefore ambiguous— results (Giner-Chavez, 1996; Schofield *et al*., 2001).

Practical use of tannins Treatments to protect dietary protein from ruminal degradation

One of the basic goals of protein nutrition in ruminants is to optimise dietary protein use in order to maximize animal growth and milk production per unit of protein consumed (Schwab, 1995). As mentioned several times, tannins could protect dietary proteins from ruminal degradation. With respect to HT, Driedger and Halfield (1972) managed to reduce the *in vitro* ruminal protein degradability of soybean meal through treatment with tannic acid. Its effect on intestinal digestibility however, was not very consistent. Terril *et al*. (1992) observed that the CT of quebracho provoked a greater reduction in the degradability of soybean meal than commercial tannic acid, but in general the results obtained were very variable and depended on many factors. One of the drawbacks of using tannins as additives to protect protein rich feeds is the possibility of their degradation by rumen microorganisms. If this were to happen, the treated feeds would be just as vulnerable to ruminal degradation as untreated feeds. However, more research is needed in this area since HT are easily hydrolysed and their effect could easily be nullified by the rumen microbiota.

Although somewhat obvious, it is worth pointing out that proper management of natural tannin-containing resources (e.g., selective grazing or supplementing the diet with the right kind of shrubs) could provide the same beneficial effects with respect to protein degradation.

Bloat prevention

It is well documented that bloat occurs when grazing ruminants consume large quantities of leguminous plants (e.g., alfalfa or clover). The gases produced in the rumen during fermentation cannot be released in the normal way since they are trapped in a persistent foam caused by the rapid release of soluble proteins during chewing and ruminal degradation. However, when these animals graze on leguminous plants containing CT (for example *Onobrychis viciifolia*) this does not occur (McMahon *et al*., 2000). The substitution of a small amount (approximately 10%) of ingested alfalfa DM by *Onobrychis viciifolia* provides unquestionable benefits in the prevention of bloat (McMahon *et al*., 1999 and 2000). The problem of this strategy is, however, the low persistence of this plant species in mixed cropping with alfalfa. The possibility of genetically modifying alfalfa to produce CT has been suggested on several occasions and has been the subject of several studies (McMahon *et al*., 2000). However, the difficulty of the molecular techniques required has made progress slow. Very recently, the preliminary results of a study on the ruminal fermentation of transgenic alfalfa were published. The *Lc* gene of maize was introduced into alfalfa to induce the synthesis of CT (Wang *et al*., 2003). The modification of the alfalfa decreased its initial rate of degradation in the rumen, but not the extent of degradation. This offers an interesting way to help to prevent bloat.

Control of internal parasites

The tannins of numerous plant species help to control certain internal parasites of animals, for example the nematode *Trichostrongylus colubriformis* (Butter *et al*., 2000). It is speculated that the positive effect on the host animal might be associated with a direct negative effect on the parasites themselves plus an indirect effect in the form of increased availability and digestive utilization of protein (Min and Hart, 2003). The literature has several examples of this in sheep and goats grazing *L. corniculatus* or *Hedysarum coronarium* (Robertson *et al*., 1995) and after having ingested quebracho CT (Butter *et al*., 2000) etc.

Essential oils

Essential oils (EO) are blends of secondary metabolites obtained from the plant volatile fraction by steam distillation (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. They are characterized as having a very diverse composition, nature, and activities. The most important active compounds are included in two chemical groups: terpenoids (monoterpenoids and sesquiterpenoids) and phenylpropanoids.

Few studies have been published on effects of EO or their constituents on milk production and composition of dairy cows. Benchaar *et al*. (2006b, 2007) observed no changes in DM intake, milk production, and milk components when dairy cows were fed 750 mg or 2 g of MEO daily. Similarly, supplementation of dairy cows with peppermint at 20 g/kg DM had no effect on milk yield and milk composition (Hosoda *et al*., 2005). More recently, Yang *et al*. (2006) observed that addition of garlic (*Allium sativa*, 5 g/day) and juniper berry (*Juniperus communis*, 2 g/day) oils to dairy cow diets had no effect on DM intake, milk production or milk composition. In these studies, the lack of effect of EO and their active components on milk performance was consistent with the absence of effects of these plant extracts on feed intake and ruminal fermentation.

EOs have an antibacterial activity against Gram-negative and Gram-positive bacteria (Helander *et al*., 1998). Several Gram-positive bacteria are involved in ruminal biohydrogenation of unsaturated dietary fatty acids (Harfoot and Hazlewood, 1988). Therefore, feeding EO could lower biohydrogenation of fatty acids by reducing the number, and the activity, of bacteria involved in the biohydrogenation of unsaturated fatty acids. Benchaar *et al*. (2007) reported no change in milk fatty acid profile when cows were supplemented daily with 750 mg of MEO. However, supplementing the same mixture at a higher concentration (*i.e*., 2 g/day) increased the concentration of conjugated linoleic acid (CLA), a health-promoting fatty acid, in milk fat.

Data on effects of EO and their compounds on beef cattle performance are almost nonexistent. In one study, Benchaar *et al*. (2006a) evaluated growth performance of beef cattle fed a silage base diet supplemented with 2 or 4 g/day of a commercial mixture of EO compounds (Vertan®, IDENA, Sautron, France) consisting of thymol, eugenol, vanillin and limonene. Results showed that DM intake and average daily gain were not affected by the addition of this EO compounds mixture. However, the gain to DM intake ratio was affected quadratically with a dose of 2 g/day maximizing feed efficiency.

References

Babu, B.J., Kalakumar, B., Reddy, K.S. and Rao, A.S. 2002. Effect of zeetress in cypermethrin induced suppression of growth and immunity in broiler. *Review of Aromatic and Medicinal Plants*, **9**: 221.

Barr, I.G., Sjolander, A. and Cox, J.C. 1998. ISCOMs and other saponin based adjuvants (Review). *Advanced Drug Delivery Reviews*, **32**: 247–271.

Batra, M. and Gupta, R. P. (2004) Effect of *ocimum sanctum* Tulsi leaf powder on clinicopathological and immune response in chickens. *Haryana Veterinarian*, **43**: 48-50.

Benchaar, C., Duynisveld, J.L., and Charmley, E. 2006a. Effects of monensin and increasing dose levels of a mixture of essential oil compounds on intake, digestion and growth performance of beef cattle. *Canadian Journal of Animal Science*, **86**: 91–96.

Benchaar, C., Petit, H.V., Berthiaume, R., Ouellet, D.R., Chiquette, and J., Chouinard, P.Y. 2007. Effects of essential oils on digestion, ruminal fermentation, rumen microbial populations, milk production, and milk composition in dairy cows fed alfalfa silage or corn silage. *Journal of Dairy Science*, **90**: 886–897.

Benchaar, C., Petit, H.V., Berthiaume, R., Whyte, T.D., and Chouinard, P.Y. 2006b. Effects of addition of essential oils and monensin premix on digestion, ruminal fermentation, milk production and milk composition in dairy cows. *Journal of Dairy Science*, **89**: 4352–4364.

Bhattacharya, A., Ghosal, S. and Bhattacharya, S. K. 2000. Antioxidant activity of tannoid principles of *Emblica officinalis* (amla) in chronic stress induced changes in rat brain. *Indian Journal of Experimental Biology*, **38**: 877-880.

Butter, N.L., Dawson, J.M., Wakelin, D. and Buttery, P.J. 2000. Effect of dietary tannin and protein concentration on nematode infection (*Trichostrongylus colubriformis*) in lambs. *Journal of Agricultural Science*, **134**: 89-99.

Cheeke, P.R. 1996. Biological effects of feed and forage saponins and their impact on animal production. In Saponins Used in Food and Agriculture, pp. 377–386 (GR Waller and Y Yamasaki, editors). New York: Plenum Press.

Coulter, A., Wong, T.Y., Drane, D., Bates, J., Macfarlan, R. and Cox, J. 1998. Studies on experimental adjuvanted influenza vaccines: comparison of immune stimulating complexes (Iscoms (TM)) and oil-in-water vaccines. *Vaccine*, **16**: 1243–1253.

Dash, D.K., Yeligar, V.C., Nayak, S.S., Ghosh, T., Rajalingam, D., Sengupta, P., Maiti, B.C. and Maity, T.K. 2007. Evaluation of hepatoprotective and antioxidant activity of Ichnocarpus frutescens (Linn.) R. Br. on paracetamol induced hepatotoxicity in rats. *Trop. J. Pharma. Res*., **6**: 755-765.

De Oliveira, C.A.C., Perez, A.C., Merino, G., Prieto, J.G. and Alvarez, A.I. 2001. Protective effects of Panax ginseng on muscle injury and inflammation after eccentric exercise. *Comparative Biochemistry and Physiology*, **130**C: 369–377.

Driedger, A. and Hatfield, E. 1972. Influence of tannins on the nutritive value of soybean meal for ruminants. *Journal of Animal Science*, **34**: 465-468.

Emadi, M. and Kermanshahi, H. 2006. Effect of turmeric rhizome powder on performance and carcass characteristics of broiler chickens. *International Journal of Poultry Science*, **5**: 1069-1072.

Gershenzon, J. and R. Croteau. 1991. Terpenoids. Pages 165–219 in Herbivores: Their Interactions with Secondary Plant Metabolites. Vol. 1. G. A. Rosenthal, and M. R. Berenbaum, ed. Academic Press, San Diego, CA.

Giner-Chávez, B.I. 1996. Condensed tannins in tropical forages. Doctoral Thesis. Cornell University. Ithaca, NY, USA.

Gupta, G. and Charan, S. 2007. Exploring the potentials of *ocimum sanctum* (Shyama tulsi) as a feed supplememt for its growth promoter activity in broiler chickens. *Indian Journal of Poultry Science*, **42**:140-143.

Hagerman, A.E. and Butler, L.G. 1991. Tannins and lignins. In: Herbivores: their interactions with secondary plant metabolites, Vol I: The chemical participants, (Rosenthal G.A. and Berenbaum M.R., eds.), Academic Press, NY (USA), pp. 355-388.

Harborne, J.B. 1999. An overview of antinutritional factors in higher plants. In: Secondary plants products. Antinutritional and beneficial actions in animal feeding (Caygill J.C. and Mueller-Harvey I., eds.). Nottingham Univ Press, UK, pp. 7-16.

Harfoot, C.G. and Hazlewood, G.P. 1988. Lipid metabolism in the rumen. In: Hobson, P.N. (Ed.), The Rumen Microbial Ecosystem. Elsevier Applied Science Publishers, London, UK, pp. 285–322.

Haridas, V., Arntzen, C.J. and Gutterman, J.U. 2001. Avicins, a family of triterpenoid saponins from Acacia victoriae (Bentham), inhibit activation of nuclear factor-kappa B by inhibiting both its nuclear localization and ability to bind DNA. Proceedings of the *National Academy of Sciences* USA **98**: 11557–11562.

Helander, I.M., Alakomi, H.-L., Latva-Kala, K., Mattila-Sandholm, T., Pol, L., Smid, E.J., Gorris, L.G.M., and von Wright, A. 1998. Characterization of the action of selected essential oil components on Gram negative bacteria. *Journal of Agriculture and Food Chemistry*, **46**: 3590–3595.

Hindustani, S.C. and Singh, K. C. P. 2006. Immunomodulatory effect of zeetress in IBD vaccinated broiler chicken. *Indian Veterinary Journal*, **83**:711-715.

Hosoda, K., Nishida, T., Park,W.Y. and Eruden, B. 2005. Influence of *Menthaxpiperita* L. (peppermint) supplementation on nutrient digestibility and energy metabolism in lactating dairy cows. *Asian-Australian Journal of Animal Science*, **18**: 1721–1726.

Hussain, I. and Cheeke, P.R. 1995. Effect of *Yucca scidigera* extract on rumen and blood profiles of steers fed concentrate- or roughage- based diets. *Animal Feed Science and Technology*, **51**: 231–242.

Jain, S. K. and Khurdiya, D. S. 2004. Vitamin C enrichment of fruit juice based ready-to-serve beverages through blending of Indian gooseberry *(Emblica officinalis Gaertn*.) juice. *Plant Foods for Human Nutrition*, **59**: 63-66.

Jie, Y.H., Cammisuli, S. and Baggiolini, M. 1984. Immunomodulatory effects of Panax ginseung C. A. MEYER in the mouse. *Agents and Actions*, **15**: 386–391.

Khanna, A. K., Ramesh, C. and Kapoor, N. K. 1996. *Terminalia arjuna*: an Ayurvedic cardiotonic regulates lipid metabolism in hyper lipidaemic rats. *Phytotherapy Research*, **10**: 663-665.

Killeen, G.F., Madigan, C.A., Connolly, C.R., Walsh, G.A., Clark, C., Hynes, M.J., Timmins, B.F., James, P., Headon, D.R. and Power, R.F. 1998. Antimicrobial saponins of Yucca Schidigera and the implications of their in vitro properties for their *in vivo* impact. *Journal of Agricultural and Food Chemistry*, **46**: 3178–3186.

Kurkure, N. V., Pawar, S. P., Kognole, S. M., Bhandarkar, A. G., Ganorkar, A.G. and Kalorey, D.R. 2000. Amellorative effect of turmeric (*Curcuma longa*) in induced aflatoxicosis in cockerels. *Indian Journal of Veterinary Pathology*, **24**: 26 – 28.

Lacaille-Dubois, M.A., Hanquet, B., Cui, Z.H., Lou, Z.C. and Wagner, H. 1999. A new biologically active acylated triterpene saponin from Silene fortunei. *Journal of Natural Products*, **62:** 133–136.

Lanjewar, R. D., Zanzad, A. A., Ramteke, B. N. and Deshmukh, G.B. 2008. Effect of dietary supplementation of tulsi (*Ocimum sanctum*) leaf powder on the growth performance and serum lipid profile in broilers. *Indian Journal of Animal Nutrition*, **25**: 395-397.

Lather, D., Mishra, S. K., Prasad, M., Gera, S., Meenakshi and Agarwal, V.K. 2002. Effect of neem (*Azadirachta indica*) seed cake feeding on immunological response of broiler chickens. *Journal of Immunology and Immunopathology*, **4**: 47-50.

Lu, C.D. and Jorgensen, N.A. 1987. Alfalfa saponins affect site and extent of nutrient digestion in ruminants. *Journal of Nutrition*, **117**: 919–927.

Mader, T.L. and Brumm, M.C. 1987. Effect of feeding sarsasaponin in cattle and swine diets. *Journal of Animal Science*, **65:** 9–15.

Makkar, H.P.S. and Becker, K. 1996. Effect of Quillaja saponins on in vitro rumen fermentation. In Saponins Used in Food and Agriculture, pp. 377–386 [GR Waller and Y Yamasaki, editors]. New York: Plenum Press.

Mamta and Mishra, S. K. 2006. Effect of tulsi (*Ocimum sanctum*) dry leaf powder on immune responses of broiler chickens experimentally infected with *Salmonella gallinarum. Journal of Immunology and Immunopathology*, **6**: 36-40.

Manu, K.A. and Kuttan, G. 2009. Immunomodulatory activities of Punarnavine, an alkaloid from *Boerhaavia diffusa*. *Immunopharmacology and Immunotoxicology*, **31**: 377-387.

Mcleod, M.N., 1974. Plant tannins - Their role in forage quality. *Nutrition Abstract and Reviews*, **44**: 803-812.

Mcmahon, L.R., Majak, W., Mcallister, T.A., Hall, J.W., Jones, G.A., Popp, J.D. and Cheng, K.J. 1999. Effect of sainfoin on *in vitro* digestion of fresh alfalfa and bloat in steers. *Canadian Journal of Animal Science*, **79:**203-212.

Mcmahon, L.R., Mcallister, T.A., Berg, B.P., Majak, W., Acharya, S.N., Popp, J.D., Coulman, B.E., Wang, Y. and Cheng, K.J. 2000. A review of the effects of forage condensed tannins on ruminal fermentation and bloat in grazing cattle. *Canadian Journal of Plant Science*, **80**: 469-485.

Michels, M.G., Bertolini, L.C.T, Esteves, A.F., Moreira, P. and Franca, S.C. 2011. Anticoccidial effects of coumestans from *Eclipta alba* for sustainable control of *Eimeria tenella* parasitosis in poultry production. *Veterinary Parasitology*, **177**: 55-60.

Min, B.R. and Hart, S.P. 2003. Tannins for suppression of internal parasites. *Journal of Animal Science*, **81**: E. Suppl. 2, E102-E109.

Mowat, A.M., Smith, R.E., Donachie, A.M., Furrie, E., Grdic, D. and Lycke, N. 1999. Oral vaccination with immune stimulating complexes. *Immunology Letters*, **65**: 133–140.

Mueller-Harvey I. and Mcallan, A.B., 1992. Tannins. Their biochemistry and nutritional properties. In: Advances in plant cell biochemistry and biotechnology, Vol. 1 (Morrison I.M., ed.). JAI Press Ltd., London (UK), pp. 151-217.

Mueller-Harvey, I. 1999. Tannins: their nature and biological significance. In: Secondary plants products. Antinutritional and beneficial actions in animal feeding (Caygill J.C. and Mueller-Harvey I., eds.). Nottingham Univ Press (UK), pp. 17-70.

Newbold, C.J., El Hassan, S.M., Wang, J., Ortega, M.E. and Wallace, R.J. 1997. Influence of foliage from African multipurpose trees on activity of rumen protozoa and bacteria. *British Journal of Nutrition*, **78:** 237–249.

Nosal'ova, G., Mokry, J. and Hassan, K. M. 2003. Antitussive activity of the fruit extract of *Emblica officinalis Gaertn*. (Euphorbiaceae). *Phytomedicine*, **10**: 583-589.

Oda, K., Matsuda, H., Murakami, T., Katayama, S., Ohgitani, T. and Yoshikawa, M. 2000. Adjuvant and haemolytic activities of 47 saponins derived from medicinal and food plants. *Biological Chemistry*, **381**: 67–74.

Odenyo, A.A., Osuji, P.O. and Karanfil, O. 1997. Effects of multipurpose tree (MPT) supplements on ruminant ciliate protozoa. *Animal Feed Science and Technology*, **67**: 169–180.

Panda, S., and Kar, A. 2003. Fruit extract of *Emblica officinalis* ameliorates hyperthyroidism and hepatic lipid peroxidation in mice. *Pharmazie*. **58**: 753-755.

Plohmann, B., Bader, G., Hiller, K. and Franz, G. 1997. Immunomodulatory and antitumoral effects of triterpenoid saponins. *Pharmazie*, **52:** 953–957.

Raghavan, B. and Kumari, S. K. 2006. Effect of *Terminalia arjuna* stem bark on antioxidant status in liver and kidney of alloxan diabetic rats. *Indian Journal of Physiology and Pharmacology*, **50**:133-142.

Rajak, S., Banerjee, S .K., Sood, S., Dinda, A. K., Gupta, Y. K., Gupta, S. K. and Maulik, S. K. 2004. *Emblica officinalis* causes myocardial adaptation and protects against oxidative stress in ischemic-reperfusion injury in rats. *Phytotherapy Research*, **18**: 54-60.

Rao, N. R., Pande, C. B. and Kiran (1999) Effect of zeetress on broiler performance- A study. *Pashudhan* **14**: 4.

Rao, T. P., Sakaguchi, N., Juneja, L. R., Wada, E. and Yokozawa, T. 2005. Amla (*Emblica officinalis Gaertn*.) extracts reduce oxidative stress in streptozotocin-induced diabetic rats. *Journal of Medicinal Food*, **8**: 362-368.

Rege, N. N., Thatte, U. M. and Dahanukar, S. A. 1999. Adaptogenic properties of six rasayana herbs used in Ayurvedic medicine. *Phytotherapy Research*, **13**: 275-291.

Robertson, H.A., Niezen, J.H., Waghorn, G.C., Charleston, W.A.G. and Jinlong, M. 1995. The effect of six herbages on liveweight gain, wool growth and faecal egg count of parasitised ewe lambs. *Proceeding of New Zealand Society of Animal Production*, **55**: 199-201.

Sadekar, R.D., Kolte, A.Y., B.S. Barmase, B.S. and Desai, V.F. 1998. Immunopotentiating effects of *Azadirachta indica* (Neem) dry leaves powder in broilers, naturally infected with IBD virus. *Indian Journal of Experimental Biology*, **36**: 1151-1153.

Sancheti, G., Jindal, A., Kumari, R., and Goyal, P. K. (2005) Chemopreventive action of *Emblica officinalis* on skin carcinogenesis in mice. *Asian Pacific Journal of Cancer Prevention*, **6**: 197-201.

Scartezzini, P., Antognoni, F., Raggi, M. A., Poli, F. and Sabbioni, C. 2006. Vitamin C content and antioxidant activity of the fruit and of the Ayurvedic preparation of *Emblica officinalis* Gaertn. *Journal of Ethnopharmacology*, **104**: 113-118.

Schofield, P., Mbugua, D.M. and Pell, A.N. 2001. Analysis of condensed tannins: a review. *Anim Feed Science and Technology*, **91**: 21-40.

Schwab, C.G. 1995. Protected proteins and amino acids for ruminants. In: Biotechnology in animal feeds and animal feeding (Wallace R.J. and Chesson A., eds.). V.C.H. Press, Weinhein (Germany), pp. 115-141.

Selvakumar, D., S.D. Rathinaswamy, S. Ramasundaram, M. Sundaramahalingam and Ramasundaram, T. 2007. Protective effect of Triphala on cold stress-induced behavioral and biochemical abnormalities in rats. Yakugaku Zasshi., 127: 1863- 1867.

Sen, S., Makkar, H.P.S. and Becker, K. 1998. Alfalfa saponins and their implication in animal nutrition. *Journal of Agricultural and Food Chemistry*, **46**: 131–140.

Sivalokanathan, S., llayaraja, M. and Balasubramanian, M. P. 2006 Antioxidant activity of *T. arjuna* bark extract on N – nitrosodiethylamine induced hepatocellular carcinoma in rats. *Molecular and Cellular Biochem*istry, **281**: 87-93.

Sjolander, A., Cox, J.C. and Barr, I.G. 1998. ISCOMs: an adjuvant with multiple functions (Review). *Journal of Leukocyte Biology*, **64**: 713–723.

So, H.S., Yoon, H.S., Kwoon, Y.S., Sung, J.H., Lee, T.G., Park, E.N., Cho, H.S., Lee, B.M., Cho, J.M. and Ryu, W.S. 1997. Effect of a novel saponin adjuvant derived from Quillaja saponaria on immune response to recombinant hepatitis B surface antigen. *Molecular Biology of Cells*, **7**: 176–186.

Soni, K. B., Rajan, A. and Kuttan, R. 1992. Reversal of aflatoxin induce liver damage by turmeric and curcumin. *Cancer letters*, **66**: 115 – 121.

Staprans, I., Rapp, J.H., Pan, X.M., Kim, K.Y. and Feingold, K.R. 1994. Oxidized lipids in the diet are a source of oxidized lipid in chylomicrons of human serum. *Arteriosclerosis and Thrombosis*, **14**: 1900–1905.

Sultan, S. I. 2003. The effect of *Curcuma longa* (Turmeric) on overall performance of broiler chickens. *International Journal of Poultry Science*, **2**: 351-353.

Terril, T.H., Rowan, A.M., Douglas, G.B. and Barry, T.N. 1992. Determination of extractable and bound condensed tannin concentrations in forage plants, protein concentrate meals and cereal grains. *Journal of Science and Food Agriculture*, **58**, 321-329.

Uko, O. J. and Kamalu, T. N. 2006. Protein quality and toxicity of full fat neem (*Azadirachta indica* A. Juss) seed kernel. *Archivos de Zootecnia*, **55**: 51-62.

Uko, O. J. and Kamalu, T. N. 2005. Haematological parameters and weight changes of cockerels fed raw or autoclaved neem seed kernels in diets. *Nigerian Veterinary Journal*, **26**: 10-17.

Uko, O. J., Kamalu, T. N., Pindiga, U. H. and Rabo, J. S. 2006. Studies on toxicity to cockerel chicks of raw full fat neem (Azadirachta indica A. Juss) seed kernel. *Veterinarski Arhiv*, **76**: 135-144.

Ursini, F., Zamburlini, A., Cazzolato, G., Maiorino, M., Bon, G.B. and Sevanian, A. 1998. Postprandial plasma lipid hydroperoxides: a possible link between diet and atherosclerosis. *Free Radical Biology and Medicine*, **25**: 250–252.

Van Soest, P.J. (ed.), 1994. Nutritional ecology of the ruminant, 2nd ed. Cornell Univ Press. Ithaca, NY, USA. 476 p.

Wang, Y., Frutos, P., Gruber, M.Y., Ray, H. and Mcallister, T.A. 2003. Comparison of *in vitro* digestibility of parental and anthocyanin-containing *Lc*-transgenic alfalfa. *Proc of the 2003 Canadian Society of Animal Science Annual Meeting*. University of Saskatchewan, Saskatoon (Canada). 10-13 June, p.27.

Wolff, S.P. and Nourooz-Zadeh, J. 1996. Hypothesis: UK consumption of dietary lipid hydroperoxides – a possible contributory factor to atherosclerosis. *Atherosclerosis*, **119:** 261–263.

Yang, W.Z., Chaves, A.V., He, M.L., Benchaar, C. and McAllister, T.A. 2006. Effect of monensin and essential oil on feed intake, milk yield and composition of lactating dairy cows. *Canadian Journal of Animal Science*, **86**: 598, Abstr.

Zheljazkov, V. D., Cantrell, C. L., Tekwani, B. and Khan, S. I. 2008. Content, composition and bioactivity of the essential oils of three basil genotypes as a function of harvesting. *Journal of Agricultural and Food Chemistry*, **56**: 380-385.

Zilversmit, D.B. 1979. Atherogenesis: a postprandial phenomenon. *Circulation*, **60**: 473–485.