2. Identity and Functional Properties of Feed Additives

Feed additives play an enormously diverse role in feed manufacture and animal production. They influence feed quality, animal health and growth and the environment. There are basically 11 major groups of compounds currently being used as feed additives as shown below:

- Organic and inorganic acids
- Antioxidants
- Amino acids
- Enzymes
- Probiotics and prebiotics
- Flavours
- Colouring agents
- Emulsifiers
- Phytochemicals
- Mycotoxin binders
- Coccidiostats and histomonostats

**Organic and Inorganic Acids**

These compounds have a very wide range of application in that they can act upon the feed or have an effect in the animal. They are used primarily as preservatives to maintain feed quality, in silage additives, as acidity regulators to modify the pH of feeds and also as acidifiers in feeds to help modulate the gastrointestinal tract in a favourable way.

**Mode of action of organic acids**

The various organic acids and combinations of acids and their salts have been proved effective against both moulds and bacteria. Incorporating salts of organic acids in acid-based products has the advantage, that salts of organic acids are easier to handle as they are less corrosive compared to the liquid organic acids and more soluble in water than some liquid organic acids. Furthermore, salts of organic acids generally have little or no odours. This can be important in pig nutrition. Generally higher dose rates of up to 5.0 kg/tonne are used to control bacteria such as Salmonella in feeds whereas mould control can often be achieved with 0.5-2.0 kg/tonne of product. The mode of action of these various organic acids in controlling the growth of moulds and of pathogenic bacteria in feeds is not completely established. The efficacy of various acids is a combination of concentration, pKa and chelating ability. There seems in general to be a pH effect and a specific effect of the organic acid molecule. Many pathogenic bacteria grow best at pH conditions close to neutrality. Desirable bacteria such as Lactobacilli on the other hand grow well under conditions of low pH.

Organic acids have been implicated in the disruption of amino acid metabolism, of DNA synthesis and of energy metabolism in micro-organisms. Acids may lower the intracellular pH, may cause alterations in cell membrane permeability, with a disruption of substrate transport for the electron transport systems. Weak lipophilic acids such as lactic, acetic or propionic are able to pass across the cell membrane of moulds and bacteria in their undissociated state, dissociate within the cell and acidify the cell interior. The cell reacts generally to maintain a constant internal pH by removing the protons, and since much of the cell energy is expended to maintain internal pH constant, growth rate is reduced.
Salts of organic acids such as ammonium propionate, calcium propionate or sodium lactate are fully dissociated in aqueous solutions, and the concentration of the undisassociated form of the added acid is low yet these salts also have useful antimicrobial activity. Accumulation of the anion of these acids must also have a growth inhibiting effect on various micro-organisms.

Characteristics Of Organic And Inorganic Acids

Organic acids such as acetic, benzoic, butyric, citric, formic, lactic, propionic, sorbic, succinic, malic, and tartaric occur naturally in a variety of feeds and foods and some of them are widely used as preservatives in feeds. Fermentation of lactic acid from sugars is important for the conservation of silages made from grass or cereals used for animal feeding.

Generally organic acids are small molecules with molecular weights less than 200. Acetic, butyric, formic, propionic, lactic, and propionic, are all liquids in the pure state, whereas benzoic, citric and sorbic acids are all solids.

A wide range of organic acids, their salts, phosphoric acid, hydrochloric acid and sulphuric acid are authorized for use in animal nutrition. Some of the more common ones are:

- **Benzoic acid**: Colourless crystalline powder which is poorly soluble in water. It is an effective antimicrobial agent and widely used in human food applications. It is only authorized for use in the EU as a zootechnical additive to improve gastrointestinal health in pigs.

- **Formic acid**: Colourless, extremely corrosive liquid, with an aggressive pungent odour in the pure state. It is readily miscible with water. It has a skin irritating effect and is the active agent in stinging nettles and in ant bites. Formic acid is widely used in antibacterial products for use in animal nutrition to control Salmonella. Not very effective against moulds, particularly at low dose rates.

- **Fumaric acid**: White crystalline, free-flowing powder, moderately hygroscopic and has poor solubility in water. At 40°C only 1.5 g dissolves in 100 g of water. It is extensively used in acidifier products for animal nutrition.

- **Lactic acid**: Odourless corrosive liquid, usually supplied in concentrations of 50 or 90%. It is usually obtained by fermentation and so is completely natural. It imparts a mild acid flavour. The pH of a 1% solution is 2.3. It has been widely used to control scouring in piglets and is an effective decontamination agent for meat.

- **Phosphoric acid**: is an inorganic acid, although frequently considered together with the organic acids. It is an odourless corrosive liquid in the pure state. The pH of phosphoric acid is much lower than the organic acids. It is very miscible with water and forms a corrosive solution. It is not a very effective antimicrobial.

- **Propionic acid**: Is a liquid in the pure state with a very pungent odour and is extremely corrosive. It is very water soluble. It is the most effective of all the organic acids as a mould inhibitor and is widely used in animal feeds.

- **Sorbic acid**: This is a white powder in the pure state. It is slightly soluble in water. However it is a very effective antimicrobial agent. It is also one of the most expensive of the organic acids and this limits its use in animal feeds.

- **Formaldehyde** is sometimes used as an antimicrobial feed additive, usually combined with an organic acid. Formaldehyde is a recognised antibacterial compound and will certainly reduce salmonella and *E. coli* counts in feed but are unlikely to have an effect in the animal. Also the regulatory status of formaldehyde as a feed additive is not fully resolved.
Management of feed quality (preservatives)

Feed materials are inevitably, and always ideal substrates for the growth of micro-organisms, both moulds and bacteria. Contamination of foodstuffs by micro-organisms causes three main problems. Micro-organisms use the nutrients in food materials for their own growth and reduce the amount available for the animal or human consumer. They may cause spoilage of foodstuffs sufficient to make them unsaleable and inedible. They may contaminate the food materials with toxic metabolites, mycotoxins, or with pathogenic micro-organisms such as Salmonella, Clostridia, E. coli, Campylobacter or Listeria. Therefore a major concern in both the human food and animal feed industries is to seek to control these micro-organisms and to minimise the damage they cause.

Because moulds can grow at lower moisture levels than can bacteria, mould contamination is a major problem for the storage of feed raw materials such as cereal grains, and oilseed meals. Low moisture feed raw materials and normally stored for some considerable time at ambient temperatures in the presence of oxygen which are suitable conditions for mould growth.

Mould contamination results in a loss of nutritive value since moulds use the raw materials for their own growth. Mould growth changes the physical properties of the raw materials because moulds produce water and generate heat during growth. Grain in silos may bridge and be difficult to move. This is extremely important when dealing with large volumes of cereals which must be loaded onto ships or lorries with automatic transporting systems.

Some moulds also have the ability to produce mycotoxins which have serious adverse effects upon growth rate and health of humans and animals. Since some mycotoxins, the aflatoxins, are carcinogenic there is obvious serious concern that such mycotoxins do not get into human foodstuffs. Most countries have very strict limits on aflatoxin contamination of animal feedstuffs and of human foods.

In the EU for example, there are strict regulations on the amount of aflatoxin that may be present in feeds published in Directive 2002/32/EC. All feed materials must contain less than 0.02 ppm (20 ppb) of aflatoxin B1 and complete feed for dairy animals must contain less 0.005 ppm (5ppb).

Concern about mycotoxin contamination of feeds has led to the addition of a functional group to the Technological Additives. This is group (m) Substances for reduction of the contamination of feed by mycotoxins.

Mycotoxin-producing fungi grow on staple raw materials for animal feeds. Products such as eggs, milk, dairy products and meat can be contaminated by animals consuming feed containing mycotoxins. Therefore mycotoxins must be considered persistent threats to the quality of feed and foods. The occurrence of mycotoxins in feeds and human foods is an animal and public health problem of major concern worldwide. Extreme vigilance and careful management is vital to ensure that levels of mycotoxins are kept acceptably low.

Therefore the first stage in control of feed quality is to reduce the risk of mould growth as mycotoxins are synthesized by moulds. Various organic acid-based products are widely used as feed preservatives to control both moulds and bacteria contamination of feeds.

Management Of The Gastrointestinal (GI) Tract :Acidifiers

The GI tract is the biggest organ in the body and requires a significant amount of nutrients for health and maintenance. The GI tract also has an enormous population of many different micro-organisms. In many species over 90% of the total cells in the body are present as bacteria in the GI tract (Gibson and McCartney, 1998). Some of these are pathogens such as species of Salmonella, E. coli and Clostridia, some are beneficial such as Lactobacilli and Bifidobacteria. Therefore it is extremely important that this population is managed for the benefit of the animal. Good health may be defined as the situation when the animal is in
control of the GI tract. Poor health may be defined as the situation when the GI tract is in control of the animal.

Previously a major use of antibiotic growth promoters was to modify the microbial population of the GI tract. Now in the absence of antibiotic growth promoters, control of the GI tract must be achieved by nutritional means through the application of various feed additives. Fortunately many feed additives, directly or indirectly, may influence the microflora in the GI tract. These include organic acids, probiotics, prebiotics and phytochemicals.

Organic acid-based products have long been extensively used in piglet feeds (Partanen and Mroz, 1999) and in calf feeds but now there is increasing interest in their application of organic acids in poultry production (Lückstädt, 2007). Acidifiers have several valuable properties including promoting animal health and performance and controlling salmonella and E.coli.

The incorporation of various organic acids into piglet feeds is now widely practised in order to help piglets adapt to the abrupt changes imposed on them at weaning. Addition of acidifiers to piglet feeds also improves the solubility and digestion of feed ingredients. The presence of acidifiers in the diet may have a positive influence on intermediary metabolism by reducing the production of toxic polyamines (cadaverine and putrescine) and of ammonia in the ileum and caecum. The polyamines and ammonia are produced from catabolism of amino acids. Therefore supplementation of piglet feeds with acidifiers could be expected to help amino acid digestibility.

Organic acids have traditionally been less widely used in poultry feeds than in pig feeds, although they are fairly commonly used in breeder feeds for control of salmonella as a part of the feed hygiene programme. All feeds in the EU are required to be salmonella-free and organic acids play an important role here. A major problem of broilers today is the presence of Campylobacter spp. which causes serious public health problems. Unfortunately Campylobacter is not feed-borne and organic acids have little effect against this pathogen in the bird.

Formic and propionic acid-based products have been extensively used as silage additives to assist the fermentation process and inhibit mould growth. The formation of silage is basically a fermentation by Lactobacilli spp. generating lactic acid. Cattle will consume a significant amount of lactic acid in silage, and rumen fermentation produces several other organic acids chiefly propionic, acetic and butyric acids. Therefore organic acids play a very important role in ruminant metabolism. However organic acid additives are not extensively used in ruminant feeds except perhaps as part of a feed hygiene programme.

The organic acids have relatively little application in petfood as dogs apparently do not find propionic acid very agreeable. Aquaculture feeds also tend to use little organic acid–based products.

**Products And Suppliers**

There are an enormous number of organic acid-based mould inhibitors, salmonella inhibitors, acidifiers and silage additives on the market. Organic acids also are the largest volume of feed additives in use. The dose rate of organic acid based products varies from 0.-5.0 kg/tonne of feed.

A major supplier of propionic acid in the EU is BASF, while formic acid is produced by Kemira, and lactic acid is produced by Galactic and Purac. Many established feed additive companies produce various mixtures of organic acids and their salts as mould inhibitors, bacterial inhibitors and as acidifiers. Some examples are; Alltech, Biomin, Kemin, Kemira, Nutriadd, and Perstop.
Antioxidants and autoxidation

Oxidation is an extremely important process in the normal metabolism of animals. Nutrients obtained from food are oxidised in a carefully controlled manner where oxygen is consumed by the body tissues to generate heat and to release energy for metabolic processes.

Paradoxically, whilst oxygen is essential for the metabolism, growth and life of animals and plants, it is also inherently dangerous to their existence because many different uncontrolled oxidation reactions, usually termed autoxidation, occur which results in the destruction of important molecules in feeds and also results in the damage of cellular tissues in animals.

It is also perhaps interesting to point out in this age of concern for so-called “natural products and programmes” that there is nothing more natural than oxidation. Autoxidation is a completely natural process in that it occurs without the intervention of man or the mediation of an enzyme. Nevertheless, autoxidation is such a destructive process, that in order to survive and prosper in such an unfriendly oxygen-rich environment we have developed the application of a variety of antioxidant molecules to control oxidative stress. Living organisms have evolved strategies using antioxidants to prevent oxidation of the contents of living cells.

Antioxidants are molecules which, at concentrations much lower than an oxidisable substrate, significantly delay or prevent its oxidation. Antioxidants are important in the production and conservation of feed raw materials and in manufactured feeds for animals.

Oxidation in feeds

There are many lipid or fat components of feeds which spontaneously react with atmospheric oxygen and suffer deterioration in the process of autoxidation. These include; fats and oils, mono- and diglycerides, and sterols. Vitamins A, D, E. and K. are fat-soluble and sensitive to autoxidation. Carotenoid pigments, lutein, zeaxanthin, astaxanthin are important in colouring poultry and fish. Carotene carotenoids are important lipid components of silages and hay and give colour to dairy products. Essential oils such as orange, peppermint or anise are used in many flavours which are incorporated into animal feeds. Emulsifiers, which may be various lecithins and lysolecithins are used in many manufactured feeds and are often incorporated into piglet and fish feeds and into calf milk replacers.

The autoxidation of feed lipid components is a major cause of reduction in feed quality, affecting nutritive value, taste, aroma, colour and texture. It also generates by-products thought to be detrimental to health. Autoxidation is a perennial danger for feeds in that it can occur at all stages; from storage of raw materials to the manufacture, storage and distribution of the feed.

Fats and oils together with other lipid materials are important and also relatively expensive components of animal feeds. Fats and oils are the greatest contributors of energy per unit of weight of any feed ingredient. The widespread use of fats and oils in animal feeds has enabled the formulation of high-energy diets for poultry, pigs and ruminants. This has undoubtedly played a major role in the improvements of productivity and animal performance seen over the last 50 years.

The total lipid components of food cover a wide range of different molecules from triglycerides, free fatty acids, xanthophylls, carotenes, vitamins, and phospholipids. They all have one common characteristic however which is the presence of long chains of carbon atoms in the molecule connected by a number of double bonds. This makes the lipids in general susceptible to deterioration through the processes of oxidation. Oxidised lipids in general lose their desirable nutritional characteristics. Fats, oils and flavours become rancid and unpalatable, vitamins lose their biological activity and pigments lose their colours.

Fat oxidation is initiated and propagated by various free radicals which are very reactive molecules. In general the oxidisable substrate is converted into an alkyl radical by proton or electron abstraction. Metal
catalysed production of radicals is by far the most important reaction in this phase. Cobalt, copper, iron and manganese are the most important metals for the initiation of autoxidation. Free radicals may also be generated under the influence of enzymes, light or heat.

The autoxidation process has three phases: initiation, propagation and termination. The time period in which the initiation phase takes place is known as the induction period. The length of this period before the onset of the propagation phase differs amongst different fats and with the degree of oxidative stress on the fat. The second phase, propagation, occurs when a critical level of free radicals react with oxygen to generate a chain reaction and produce alkyl peroxides. The peroxides react further with new substrate molecules to generate hydroperoxides and more free radicals. The propagation phase is characterised by rapid consumption of oxygen and the generation of heat which in extreme cases can destroy the feed materials and even ignite materials such as fish meal which has a significant oil content. The third phase, termination, comprises the recombination of various species of free radicals to produce stable end products. These are a wide variety of hydrocarbons, aldehydes, ketones, alcohols and organic acids. This has the effect of slowing down oxidation and it also generates characteristic unpalatable or rancid flavours and odours, which makes food unacceptable to the consumer.

Factors which influence autoxidation of lipids
The oxidative status of feed lipids is influenced by several factors such as environment, presence of metals and enzymes.

- **Moisture:** If fats and oils contain more than 0.1% moisture, hydrolysis of triglycerides can occur generating free fatty acids which are easily oxidised.
- **Temperature:** As with all chemical reactions, increasing temperature increases the rate of autoxidation. However cooling below ambient temperature will not necessarily prevent oxidation as it can occur at low temperature.
- **Light:** Light provides a source of energy which may promote the oxidation of fats and oils. Ultra violet light is the most effective. Photo-oxidation is much more rapid than classical autoxidation.
- **Metals:** Copper, manganese, and iron are very effective catalysts which promote free radical formation leading to oxidation of fats and oils.
- **Air:** Exposure to air because of its oxygen content should be kept to a minimum. A peroxide value of 20 meq / kg fat can be generated by the fat absorbing only 0.016% of its own weight of oxygen.
- **Enzymes:** Fat metabolising enzymes lipases and lipoxygenases occur in many plant seeds which are used in feeds. Lipase enzymes split free fatty acids from triglycerides and promote autoxidation. Lipoxygenase catalyses the direct addition of oxygen to unsaturated fatty acid molecules producing hydroperoxides:

This enzyme is very widespread in plant materials, soyabees are a particularly rich source, but cereal grains and many fruits and vegetables also contain active lipoxygenase enzymes. Lipoxygenase activity is a major cause of off-flavours in raw plant foods but fortunately it is not very heat stable. Therefore heat treating of feed ingredients such as soyabean meal will reduce the problems of lipoxygenase-catalysed oxidation of lipids.

Consumption of oxidised feeds
Consumption of oxidised feeds is a potential health risk for animals. The first products of autoxidation of a fat are odourless, tasteless peroxides and hydroperoxides. Hydroperoxides are very toxic when administered
intravenously but fortunately are considerably less toxic when ingested. Possibly they are poorly absorbed from the gastrointestinal tract or they may be further metabolised in the gut.

At ambient temperatures however hydroperoxides breakdown and produce a variety of hydrocarbons, aldehydes, ketones, alcohols and organic acids. The production of these end products is the chemical manifestation of rancidity. The undesirable flavours in rancid materials can be caused by very small quantities of only a few ppm of aldehydes and ketones. Therefore the amount of lipid in a feed mixture is actually less important than its nature and susceptibility to oxidation.

Many of these stable oxidised end products such as aldehydes and ketones have a carbonyl residue in the molecule. Due to their strong hydrophilic nature and low molecular weight these components in oxidised fats and oils are easily absorbed and carried to the internal organs in the bloodstream and promote lipid oxidation in vivo.

Oxidised feeds may be consumed by animals although at a reduced rate which affects growth and performance, and this has been demonstrated in broilers (Engberg et al., 1996). At 38 days of age broilers fed oxidised oil had an average body weight 109g or some 5% lower than broilers fed fresh oil.

Nutritional studies with different animal species demonstrate that feeding oxidised fats and oils generally results in reduced feed consumption and has undesirable physiological effects. These can be reduced growth rates, reductions in body tissue levels of physiological antioxidants such as tocopherols and induction of anaemia. Consequently oxidative status of dietary fats and oils is very important for production of good quality feeds.

Antioxidants

Oxidation is such a destructive process that living organisms have evolved strategies to prevent oxidation of the contents of living cells. For our own economic well-being we have devised strategies to control oxidation in various products. Indeed control of oxidation is a major preoccupation for the feed, food, plastics and rubber industries, all of whom deal with products susceptible to oxidation.

Antioxidants have already been defined as substances which when present at much lower concentration than an oxidizable substrate, significantly delay or prevent its oxidation. Many different substances are known to have antioxidant activity, although only a relatively small number of these are routinely used as antioxidants in feeds. The list of molecules approved for use as antioxidants in feeds in the European Union contains both synthetic and natural molecules (Table 1).

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<th>Table 1. Antioxidants Permitted for Use in Foods in the European Union</th>
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<tr>
<td>L-Ascorbic acid</td>
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<td>Sodium L-ascorbate</td>
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<td>Calcium L-ascorbate</td>
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<tr>
<td>5,6 Diacetyl-L-ascorbic acid</td>
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<tr>
<td>6-Palmitoyl-L-ascorbic acid</td>
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<td>Tocopherol-rich extracts of natural origin</td>
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<td>Synthetic α-tocopherol</td>
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| Synthetic γ-tocopherol | |}

These compounds have been used for many years and have given good service. However consumers all over the world are becoming increasingly conscious of the nutritional value and safety of feed and its ingredients. At the same time there is an increased preference for natural feed ingredients which increasingly favours the use of compounds of natural origin. In addition to the specific compounds listed in Table 1, many