Bioflavonoids’ effects reach beyond productivity

Previously, Feed Mix has explored the proposed mode of action of a group of powerful antioxidants found in citrus and other fruits. As we get to know the bioflavonoids better, the potential applications in animal nutrition, specifically meat production, are becoming more widespread.

By Clemente J. López Bote

Over the past 50-60 years, the development of modern feeding practices has resulted in the wide variety of feeds of varying composition that were tailored to the local practices of the time, transformed into the more homogenised feeds and systems with which we are now familiar. All this activity was the tip of the iceberg in terms of nutritional development, however. The “scientific era” of animal feeding began in 1783 with French chemist Antoine Laurent de Lavoisier’s explanation of respiration, and became steadily more quantitative over the next 200 years. The 20th century heralded the real scientific breakthroughs, with the discovery of vitamins, insulin, fatty and amino acids and the importance of trace elements. Now, the requirements have been identified and quantified for each productive circumstance and can be balanced against their availability in feed ingredients. So far, most efforts have focused on a limited number of identified nutrients-for which we have managed to genetically select a limited number of plants that are rich in these nutrients.

Technological treatments have been developed with the ultimate aim of obtaining a favourable concentration and availability of the nutrients required from the feedingstuffs available. The result is the present high efficiency diets that achieve almost perfect synchrony between the animal’s requirements and the nutrients provided by the diet; this results in uniform production of food from animal origin. However, in doing so, many details and possible interactions have been overlooked. These include:

- the development of some pathologies
- the immune response
- regulation of feed intake
- meat quality
- oxidative status
- stress adaptation
- health status

Flavonoids- the hidden nutrients?
Bioflavonoids are a group of natural polyphenols, soluble in water, present in many fruits where they confer colour and flavour characteristics and are essential for the absorption and metabolism of vitamin C. At present, there are more than 5000 polyphenols identified and classified. They were first reported in the 1930’s by Dr Albert Szent-Gyorgi, the Hungarian scientist who won the Nobel prize for his discovery of vitamin C; he initially classed them as a vitamin (“vitamin P”, after the protective effect he found them to exert on the capillaries). However, when it became experimentally demonstrated that they were not essential (i.e. that a lack of intake does...
not induce deficiency symptoms), interest was lost. In the last 15 years, the presence of bioflavonoids has been demonstrated in many foods and feed ingredients, and their consumption has been related to some health benefits. This has attracted the attention of nutritionists in recent years regarding wine, beer, citrus fruits, cherries etc. From a nutritional point of view, our knowledge of bioflavonoids is still in its initial stages.

There are six groups of bioflavonoids, as shown in Table 1. The anthocyanidins are present in cherries, berries and grapes; the catequins are present in apples, tea and coconut; the flavons can be found in certain herbs and spices such as parsley, thyme and pepper; the flavonols include quercetin and are present in onion, apples, cherries, tea and berries; flavonons, including hesperidin and naringin, are commonly found in citrus fruits; and the isoflavons found in legumes such as soybeans. Their biological properties are related to a number of factors in vitro, including activity as antioxidants antioestrogenic properties; antiproliferative activity; antienzymatics; antimicrobials and anti-inflammatories. Clinical and epidemiological studies have shown the various bioflavonoids to play useful roles in preventing cardiovascular disease and some cancers.

All the flavonoids can be identified chemically by HPLC and can be extracted and purified for application in the chemical and pharmaceutical industries or converted for other specific applications. However, experience has shown that care should be taken when separating these substances, as this can affect their efficacy. For example, tangeretin from bergamot (Citrus aurantium L. bergamia) shows higher anticarcinogenic activity than the other 21 bioflavonoids, but only when it is derived from a natural extract—the isolated form doesn’t show this strong effect.

Some of the most studied bioflavonoids with respect to their possible applications in animal nutrition are shown in Table 2. Previous reports indicate that the bioflavonoids can exert antimicrobial effects in the intestine when administered via the feed. Beyond this, they are absorbed into the blood circulation (Donovan et al., 1999; Erlund, 2001) and are distributed throughout the organism, before being excreted in urine and sweat. Animals cannot produce bioflavonoids as plants do, therefore they have to be added to the diet to exert the numerous physiological benefits that have been investigated in human and now veterinary medicine (Table 3).

Oxidation and peroxidation
The main feature of the bioflavonoids is their powerful antioxidant capacity, which provides protection against oxidative and free radical damage.

As Lavoisier reported in 1783, life depends on the controlled oxidation of carbohydrates, fatty acids and amino acids, which release their energy in the presence of oxygen via the Krebs cycle. The main feature of the bioflavonoids is their powerful antioxidant capacity, which provides protection against oxidative and free radical damage.

<table>
<thead>
<tr>
<th>Flavons</th>
<th>Flavonols</th>
<th>Flavonones</th>
<th>Catequins</th>
<th>Anthocianins</th>
<th>Isoflavons</th>
</tr>
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<tbody>
<tr>
<td>Apigenin; luteolin</td>
<td>Quercetin; myricetin</td>
<td>Naringenin, hesperedin</td>
<td>Epicatequin; galactequin</td>
<td>Pelargonidin; malvidin</td>
<td>Genistein</td>
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<table>
<thead>
<tr>
<th>Common sources</th>
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<tbody>
<tr>
<td>Parsley, celery, pepper, thyme; Onion, apple, cherry, tea, berries; Citrus fruits, plums; Apple, tea, coconut; Cherries, grapes (wine); Legumes</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Fruit/plant</th>
<th>Latin name</th>
<th>Bioflavonoids</th>
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</thead>
<tbody>
<tr>
<td>Bergamot</td>
<td>Citrus bergamia</td>
<td>Rutin; Eriodictiol; eriodictin</td>
</tr>
<tr>
<td>Mandarin</td>
<td>Citrus reticulata</td>
<td>Rutin</td>
</tr>
<tr>
<td>Grapefruit</td>
<td>Citrus paradisi</td>
<td>Naringin; kaempferol</td>
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<tr>
<td>Sweet orange</td>
<td>Citrus sinensis</td>
<td>Hesperidin; tangeretin</td>
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<tr>
<td>Lemon</td>
<td>Citrus limon</td>
<td>Diosmetin; Disomin; Eriodictiol; eriodictin;</td>
</tr>
<tr>
<td>Hesperidin</td>
<td>Citrus aurantifolia</td>
<td>Diosmetin; Disomin;</td>
</tr>
<tr>
<td>Lim</td>
<td>Rosmarinus officinalis</td>
<td>Apigenin; Luteolin</td>
</tr>
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Figure 1. Controlled oxidation via the Krebs cycle is the basis of the cell’s energy metabolism

Figure 2 - Peroxidation by free radicals is self-perpetuating and can be very damaging

(-CH₂-CH=CH₂-CH₂-CH=CH₂)

Stress | Illness | Light; O₂ | Metals; |

(-CH₂-CH=CH₂-CH₂-C*=CH₂) Free radical

Very reactive | Toxic | Carcinogenic | Aging | Immune response | Cardiovascular disease
Krebs cycle, producing ATP (the universal currency of biological energy transfer), with carbon dioxide, water and heat as by-products (Figure 1).

Under certain circumstances, however, oxidation can become uncontrolled and a very destructive process known as peroxidation occurs. Amino acids, prostaglandins and the polyunsaturated fatty acids of cell membranes are particularly at risk from peroxidation. Stress, illness, excessive exposure to light, oxygen and certain metal ions can initiate the formation of peroxide radicals, as shown in Figure 2. Biochemical mechanisms exist to protect organisms against peroxidation, including antioxidant enzymes such as catalase, glutathione peroxidase and superoxide dismutase and other lipophilic (tocopherols, ubiquinone and the carotenoids) and hydrophilic (ascorbic acid, thiols, amines, uric acid, polyamines, amino acids, peptides and proteins) compounds. The activity of vitamin E and its interaction with vitamin C within the phospholipid bilayer of the cell membrane is a well known example of one of these protective mechanisms. However under certain levels of stress, this type of system may not be sufficient, as demonstrated by Soares and López Bote in 1999. Malonaldehyde is a commonly used indicator of the level of lipid peroxidation. This was found to rise sharply in the plasma of animals at the time of particular stressors, such as weaning. Furthermore, after slaughter, the enzyme-regulated mechanisms of protection are inactivated. Then peroxidation is unavoidable, and meat becomes unacceptable for human consumption or processing in a matter of days. This is a particular problem in a modern global market, where food can travel enormous distances before consumption.

**Meat quality**

The postmortem oxidation has been found to depend greatly on the pre-slaughter ratio of pro-oxidants to antioxidants. Often, it is of interest to try and potentiate the protective mechanisms post-mortem, by increasing the levels of chemically active antioxidants that do not rely on metabolism. One such example is to increase the tissue content of phenolic antioxidants like the bioflavonoids. These are able to “mop up” free radicals, supporting the membrane-based systems. The consequences of post-mortem oxidation on meat quality are as follows: fatty acids are oxidised to produce free radicals (which initiate peroxidation) and produce aldehydes and ketones, which cause off-odour and off-flavours and can be used as a measure of oxidation; the damage to cell membranes causes leakage of the cell contents, resulting in loss of the water holding capacity of the meat (increasing drip loss); cholesterol oxidation products can also be used as an indication of peroxidation; the oxidation of haemo pigments (the iron-containing substances that give raw meat its characteristic redness) causes a loss of colour stability. A number of studies have demonstrated that administration of increased levels of antioxidants ante-mortem via the feed can improve the oxidative stability of meat post-mortem and, hence reduce the appearance of oxidation products. Monahan et al. (1992) showed that increasing the tissue content of vitamin E by feeding 200ppm α-tocopherol before slaughter maintained oxidative stability of meat as indicated by a lack of increase of the levels of thiobarbituric acid-reactive substances (TBARS - a measure of oxidative activity) and significantly reduced the increase in drip loss until eight days post-mortem. Similarly, the levels of cholesterol oxidation products were significantly reduced in cooked pork stored for 9 days when the levels of vitamin E in the meat were increased by supplementing the diet with 200ppm vitamin E prior to slaughter.

**Bioflavonoids maintain meat quality**

The efficacy of bioflavonoids in maintaining meat quality has been indicated by a number of studies in the meat of various species. In *in vitro* studies with stored cooked chilled chicken meat, 200ppm quercetin reduced the development of oxidation products in the meat to almost zero over a period of five days (Karastogiannidou et al., 1999). *In vivo* studies seem to support the *in vitro* findings, indicating that ingestion of dietary flavonoids prevents lipid peroxidation, protecting cell and organellar membranes (Frémont et al., 1998; Rey et al., 1997). In broilers, the effects of adding 500mg/kg extracts of sage or rosemary to broiler feed on lipid oxidation in the meat was compared to a negative control (not supplemented with antioxidants) and a positive control diet containing 200mg/kg α-tocopheryl acetate López-Bote et al., 1998). After nine days of refrigerated storage, thiobarbituric acid reactive substances (TBARS) of white meat from broilers fed the negative and positive control diets were 0.51 and 0.25mg malonaldehyde/kg meat, respectively (Figure 3). Values for meat from broilers that had been fed extract of rosemary (rich in apigenin and luteolin, Table 2) or sage were found to be in the range 0.30-0.35mg malonaldehyde/kg meat, respectively, significantly lower than that from birds fed the negative control diet. A similar trend was observed in the dark meat, but the differences were not statistically significant after nine days’ storage (Figure 4). Under the more extreme conditions of frozen storage, similar trends were observed when raw samples were stored at −20°C for up to four months and in samples cooked at 70°C and stored under refrigeration for up to four days.
The meat from broilers fed the diets containing citrus bioflavonoids had smaller concentrations of oxidation products than that from the control group (P<0.05) (Figure 5).

Drip loss, pH and colour evaluation
After slaughter, when the supply of oxygen and nutrients to the muscles ceases, any subsequent metabolism must be anaerobic and ATP (for energy) can only be generated through glycolysis. As glycogen is broken down, lactic acid accumulates, acidifying the meat since there is no circulatory system to remove it (Figure 6). Typically, the pH of healthy muscle will fall from about 7.2 to around 5.5, though this final value varies depending on the muscle. As the pH falls, proteins begin to denature and lose the water that is bound to them, leading to exudation of fluid from the muscle fibres. The change in proteins also increases the light scattering properties of the contractile elements of the muscle fibre. The meat then changes from being dark and translucent to being paler and opaque. Work conducted at Iowa State University (Kremer et al., 2000) showed that supplementing the diet of pigs with a common bioflavonoid, quercetin, maintained normal pH over a longer period of time and hence improved muscle water-holding capacity (as measured by a reduction in water loss). Subsequent work with 200ppm citrus bioflavonoids (BioCitro, Probena SA, Spain) supplemented to broiler diets has shown similar reductions in drip loss in breast and leg meat (Figure 7).