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Use of Saffron (*Crocus sativus* L.) as a Feed Additive for Improving Growth and Meat or Egg Quality in Poultry

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ABSTRACT

From ancient times, saffron, the flower of the plant *Crocus sativus* L., is widely used as a traditional medicine to promote health and fight disease. *Crocus sativus* is cultivated in different parts of the world such as Greece, Spain and, mainly, Iran. In view of its wide range of medicinal uses, saffron has submitted to extensive phytochemical and biochemical studies and a variety of biologically active substances that may exhibit diverse activities have been isolated. The goal of this review is to discuss and summarize the scientific data on the biological properties of saffron and its main ingredients, and to investigate its possible use as a feed additive. The poultry industry would greatly appreciate natural antioxidants that could replace the synthetic ones and satisfy consumer demands for production of eggs and meat without residues from substances that have the potential to harm human health. With natural agents, such as saffron, the hope is that their availability, lack of obvious toxicity at effective dose and ability to protect health by various mechanisms, would allow their introduction as potential feed additives. The major challenges are the standardization of the biological multi-component composition derived from saffron and the standardization of their effects on animal performance and food quality and safety.

Keywords: *Crocus sativus*, poultry nutrition, spices

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INTRODUCTION

Saffron is the most precious spice in the world. Saffron filaments or threads are actually the dried stigmas of the flower of the plant *Crocus sativus* Linneaus. *Crocus sativus* L. is a perennial stemless herb of the *Iridaceae* family, widely cultivated in Mediterranean countries such as Greece, Spain and France, as well as in Middle East and Southeast Asian countries, especially Iran and India.

Each flower contains only three stigmas, which must be picked from each flower by hand. Commercial saffron comprises the dried red stigma with a small portion of the yellowish style attached. It is estimated that more than 75,000 of these flowers are needed to produce just one pound of saffron filaments, making it the world's most precious spice and more than 50,000 of these stigmas are needed to produce just 100 g of red saffron. Because of saffron's strong colouring power and intense flavor, it can be used sparingly. There are no references in the literature to the use of other parts of the plant, like leaves or petals, although the toxic effect of the bulb on animals has been described (Abdullaev 2004).

Saffron is among the richest spices in carotenoids and contains about 150 volatile aromatic substances. It contains crocin, the source of its strong colouring property, bitter-crocin, which offers the distinctive aroma and taste, and essential oils which are responsible for its therapeutic properties (Del Campo *et al*. 2010).

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Invited Review

Saffron is used both for its bright orange-yellow color and for its unique and distinctively pungent, honey-like flavor and aroma (Lech *et al.* 2009). The word "saffron" is derived from the Arabic word zafaran, which means "yellow." The French culinary term safrané means 'colored using saffron'. In India, its color is considered the epitome of beauty and it is the official color of Buddhist robes.

It is most commonly used as a food colouring and flavouring herbal agent in various regions of the world (Nair *et al.* 1991a, 1995). It is also employed in alcoholic and non-alcoholic beverages. Saffron has been used as a textile dye and extensively exploited in pharmaceutical preparaions. It has, however, been used in folk medicine for its analgesic and sedative properties, but the many therapeutical virtues attributed to it through the ages are now under discussion. Interesting reviews on the use of saffron throughout history (Folch 1957), in medicine, food and commerce (Basker and Negbi 1983), as well as, quality and agricultural production (Ordoudi and Tsimidou 2004) have been published.

Saffron is commercially available both in filaments and powder, though the long, deep red filaments are usually preferable to the powder, as the latter can be easily adulterated (Ordoudi and Tsimidou 2004). Today, the greatest saffron producing countries are Greece, Spain, Turkey, Iran, India, and Morocco. The largest saffron importers are Germany, Italy, U.S.A., Switzerland, U.K., and France. Greek red saffron or "Crocus", a pure product of the Greek agriculture, is considered as one of the best in the world. Greek saffron has a colouring strength of 256, verified by laboratory reports, which is 45 points higher than the minimum international standard for all saffron as declared by the Cooperative De Safran Kozani which is the producer of Greek Crocus (natural product, Protected Name of Origin) and employs the BVQI ISO 9002 procedures.

The aim of the present review is to discuss the most recent research on the pharmacological activities of saffron and its active constituents in order to verify the properties attributed to them over the centuries and to highlight its use in animal diets in order to promote both their performance and health.

SAFFRON IN MYTHOLOGY AND HISTORY

Greek mythology tells about a handsome man named Crocus who fell in love with the beautiful nymph Smilace, the favourite one of the god Hermes. The god, in order to punish Crocus, transformed him into a flower of saffron. Also, according to Greek mythology, the bed of Zeus, king of the Olympus, was spread of saffron flowers. Known since antiquity, saffron was one of the most desired and expensive spices of ancient Greeks, Egyptians and Romans for its aroma, colour and aphrodisiac properties. It was quite popular among the Phoenician traders, who carried it wherever they travelled. Ancient Assyrians used saffron for medical purposes. According to Assyrian customs, Semiramide cultivated saffron in the hanging gardens of Babylonia, one of the Seven Wonders of the World. Hippocrates and other Greek physicians, like Dioskourides and Galinos refer to crocus, as a drug or a therapeutical herb. It was mentioned by Homer in the Iliad, whereas Pliny the Elder knew its therapeutic properties as a remedy for stomach ulcers, as well as a remedy for cough and angina pectoris. Egyptians used it for dyeing fabrics, to make perfumes and unguents and, last but not least, to flavour foods (Kumar *et al.* 2009).

Saffron was not used only for cooking and it was frequently used for the production of cosmetics. In 2000 BC in Crete Island saffron was used to colour the breast and lips of women. Among the ingredients used for perfumes and colorants, saffron was one of the most important ones, and it became the main ingredient of the two most exclusive perfumes of Athens, Susinum and Crocinum. Romans, despite imperial laws forbade it, imported perfumes and essences from Greece and from other countries. In Roman thermae, rich people used to take a bath in water mixed essences and saffron. The Romans initially brought saffron to England (Kafi *et al.* 2006). Cleopatra used it as a cosmetic and to dye her skin, nails, lips and hair. In the Bible was mentioned in the "Song of Solomon" book, in the fourth chapter, associated to the most aromatic and precious essences. In ancient Jerusalem it was greatly demanded for liturgies (Rashed-Mohassel 2006).

At the end of the 1300s, the cultivation of saffron, called for many centuries "vegetal gold", was very common in Italy, Spain and Greece (Skrubis 1990). In Italy it is mainly cultivated in Abruzzo, in the upland of Navelli, in Sardinia in the production areas of saffron, in Umbria at Cascia and in Tuscany in the province of Siena. In these areas, the production of saffron is still active and, in some of them, the quality is very high. In the Middle Age it was used as a remedy against epilepsy and plague. The history of red saffron in modern Greece starts in the 17th century when traders from Kozani, Macedonia, Greece, brought the red saffron from Austria. For 300 years, Greek red saffron is systematically cultivated under the warmth of the Greek sun, in the rich soil of a unique area including many small towns of Kozani in West Macedonia, Greece.

SAFFRON PRODUCTION

C. sativus cultivation reaches back more than 3,000 years. The wild precursor of domesticated saffron crocus was *Crocus cartwrightianus*. Human cultivators bred wild specimens by selecting for unusually long stigmas. Thus, a sterile mutant form of *C. cartwrightianus*, *C. sativus*, emerged in late Bronze Age in Crete. Saffron has been used as a spice and medicine in the Mediterranean region since then, with usage and cultivation slowly spreading to other parts of Eurasia as well as North Africa and North America. The plant is also found in the Middle East countries or in the east areas of Iran, India and Kashmir. In the last several decades, saffron cultivation has even spread to Oceania and Mexico (Abdullaev 2004).

C. sativus derives from the artificial selection of the plant, because it is an intensive and artificial selection that is made by man. The plant is sterile, therefore the only way of reproduction is by cloning and separating secondary bulbs from primary ones. The bulb of the saffron plant is very sensitive to the action of parasite fungi. Some animals, such as boar and porcupine, are very greedy of the bulbs of *C. sativus*, therefore they are potentially dangerous for non-fenced plantations. Also mice are to be considered dangerous for stored bulbs waiting to be planted (Ordoudi and Tsimidou 2004).

C. sativus is cultivated in several countries with mild and dry climate. This plant likes climates with moderate rains, an altitude from 500 to 700 m above sea level and permeable and well drained soils, but it is not prosperous in rainy climates and soils retaining water. As for temperature, the plant is very tolerant. In summer, it can stand to high temperatures whereas in winter it can stand to light frosts and temperature down to -10°C (Rashed-Mohassel 2006).

Collection time is in autumn, when the flowers are opening. Flowers are picked in the early morning, and the stigmata are manually removed and dried. The quantity of flowers required to produce 1 kg of saffron varies between 150,000 and 200,000. Traditional saffron production is still mainly family-based work; although the need for good agricultural practice methods to conform production is now emerging (Ordoudi and Tsimidou 2004).

The world's total annual saffron production is estimated at 190-205 tons/year, with >80% of this harvest originating from Iran, mainly from the Khorassan province (Betti and Schmidt 2006; Hagh Nazari and Keifi 2006). Within Europe, Spain is generally believed to be the major source of cultivated *C. sativus*, based on an annual export of approximately 60 tons. However, since the collection of saffron is a very costly and time-consuming procedure, the bulk of saffron re-exported from Spain is in fact of Iranian and Moroccan origin. In fact, the largest European saffron producer is

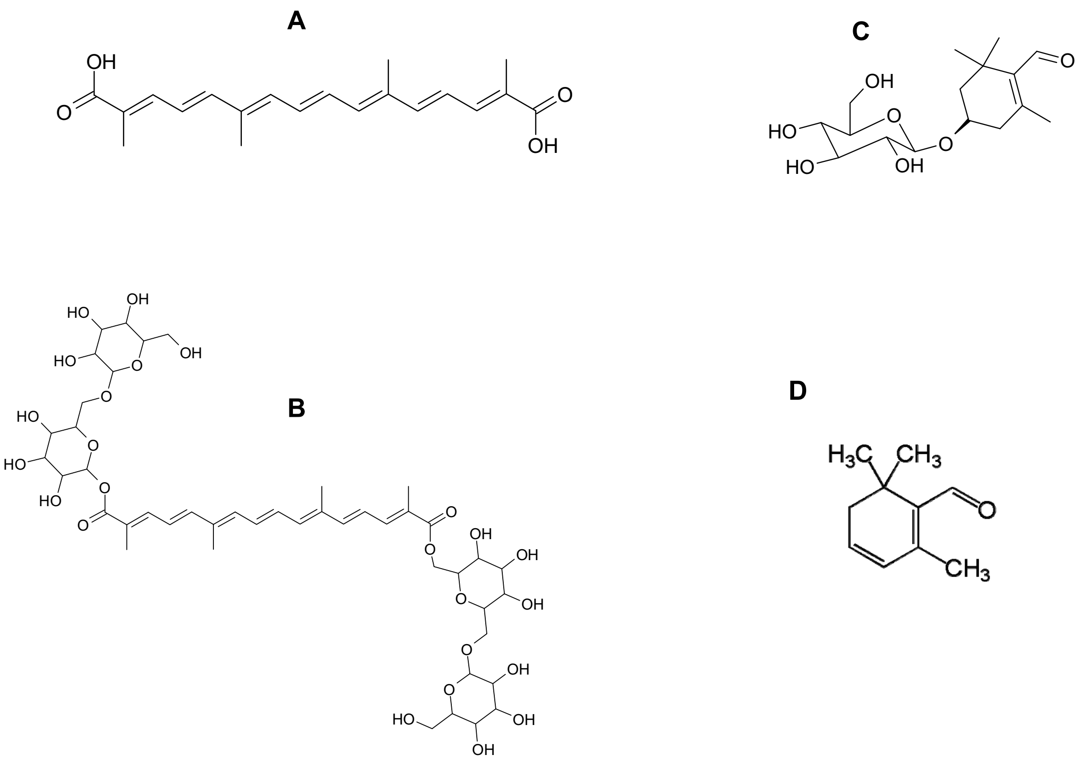

Fig. 1 Chemical structure of crocetin (A), crocin (B), picrocrocin (C), and safranal (D).

currently Greece with 4.5 tons/year, a figure which has remained stable in the past years, and by far surpasses the current Spanish production (Schmidt *et al.* 2007).

The decline of European saffron cultivation seems to be closely related to the high labour costs involved in saffron production. The re-exporting and re-labelling of saffron probably contributes to the finding of a relatively uniform quality of saffron independent of the labelled origin (Hensel *et al.* 2006). Adulterations are frequent. At least 30% of saffron samples do not fulfil quality specifications or are to be considered as waste products or even a non-saffron product (Hensel *et al.* 2006; Schmidt *et al.* 2007).

CHEMICAL COMPOSITION AND QUALITY ASPECTS OF SAFFRON

Saffron quality is characterized by its typical combination of bitter taste, aromatic smell and intense red color. Its bitter taste originates from the flavonoid derivative picrocrocin, a *β*-D-glucoside of hydroxysafranal (Wintherhalter and Straubinger 2000). The harvesting and drying process may strongly affect the taste and flavour of saffron. During processing, picrocrocin converts into the tasty safranal, 2,6,6-trimethyl-1,3-cyclohexadien1-carboxaldehyde, by thermic or enzymatic degradation (Pfander and Schutenberger 1982; Curro *et al.* 1986; Ibora *et al.* 1992). Other trace compounds, mostly monoterpenoids from the volatile fraction and secondary degradation products from non-volatile compounds additionally affect the aroma (Wintherhalter and Straubinger 2000).

Its colour originates from the crocins (**Fig. 1**), which are all glycosides of *trans*-crocetin, a carotenoid derivative, with various sugar ester moieties. Besides crocins, *cis*-crocetin and its glycosides are also present in saffron. Good quality saffron contains about 30% of crocins, 5-15% of picrocrocin and usually up to 2.5% of volatile compounds including safranal (Schmidt *et al.* 2007).

Quality control of saffron involves identifying and quantifying crocin derivatives which are the major biologically active compounds in the spice. Liquid chromatography has been widely used in the quality control of saffron because it allows the simultaneous determination of saffron components such as picrocin, its aglycone, crocin derivatives and safranal (Li *et al.* 1999; Lozano *et al.* 1999). Basically, all the studies on the chemistry of C. *sativus* refer to its stigmas, the chemical composition of which is widely known (Dhar and Suri 1974; Ruppolt 1975; Amelotti and Mannino 1977; Mannino and Amelotti 1977; Corradi and Micheli 1979a, 1979b; Corradi 1981; Corradi *et al.* 1981; Pfander and Rychener 1982). Dried stigmas of saffron should contain no more than 12% water, 7% mineral matter, 8% fat, 13% protein, 20% reducing sugars, 7% pentosans, 10% gums and dextrins, and some free sugars (Ordoudi and Tsimidou 2004).

Few studies have been done on the chemical composition of other organs of saffron, though there is one on the flavonoid content of pollen and some of petals (Song 1990) and tepals (Garrido *et al.* 1987). Kaempferol, astragalin, helichrysoside, kaempferol-3-glycopyranosyl (1-2)-6-acetylglucopyranoside, kaempferol-3-glucopyranosyl (1-2)-glucopyranoside, miricetin, quercetin, delphinidin, petunidin and delphinidin-3,5-diglucoside have been isolated or identified in different organs from C. *sativus*. Vitamins, especially riboflavin and thiamine, have been described in different samples of saffron (Sampathu *et al.* 1984), whereas various volatile compounds including 2-phenyl ethanol, naphthalene, 2-butenoic acid lactone, safranal, isophorone, six trimethyl-cyclohexyl derivatives (Sampathu *et al.* 1984; Kanakis *et al.* 2004), and the fatty acids, palmitic, stearic, oleic, linoleic and linolenic (Zarghami and Heinz 1971) have been detected in the essential oil of saffron. The saffron bulb contains glucose, aspartic and glutamic acids, cysteine, serine, glycine, threonine, tyrosine, alanine, arginine, histidine, lysine, proline, phenylalanine, leucine, valine, methionine, two saponins (one triterpenic and another steroidic) and a high molecular weight protein (Sampathu *et al.* 1984; Liakopoulou *et al.* 1985).

USE OF SAFFRON IN TRADITIONAL MEDICINE

There are many reviews on the uses of saffron in traditional medicine (Folch 1957; Grieve 1977; Consorti 1980; Basker and Negbi 1983; Hadjsharifi and Tabatabaee 1986). In general, saffron, its extracts and tinctures are used as folk remedies against cramps, asthma and bronchospasms, menstruation disorders, liver disease and pain. Saffron was also said to have a soothing and tonifying effect on the gastrointestinal tract (Zougagh *et al.* 2005). A major use, however, is the application as a stimulant, aphrodisiac and antidepressant (Ríos *et al.* 1996; Abe and Saito 2000; Ference and Bedersky 2004; Akhondzadeh *et al.* 2005).

In addition, saffron and its constituents are able to increase the uterine motility tone (Chang *et al.* 1964) by acting as an emmenagogue agent and, at higher doses, by producing metrorrhagias and even abortion. Paradoxically, it has also been reported that saffron has a uterine sedative property, which is useful in dysmenorrhoea diseases and the premenstrual syndrome (Leclerc 1983). These effects at the uterine level have dictated its use in folk medicine, but there are reports of its toxicity, and at least one death has been attributed attributed to the ingestion of 1.5 g (Basker and Negbi 1983). In an extensive review concerning the use of saffron in traditional medicine, Schmidt *et al*. (2007) concluded that saffron is used in painful menstruation, lumbar pains, atonic dyspepsias, coughs, bronchial spasms, asthma and teething problems.

The medicinal use of saffron has decreased in recent decades and its uses have been reduced to that of a dye and spice (Basker and Negbi 1983). According to Ríos *et al.* (1996) it is only used for its colouring properties, whereas for others it possesses a clear eupeptic function because it facilitates the digestive process by increasing gastric secretions. The bitter substances were considered as active compounds against several diseases in the classic books on pharmacognosy and phytotherapy. These books also refer to the use of saffron as an antispasmodic in digestive diseases in which intestinal motility is increased.

BIOLOGICAL PROPERTIES OF SAFFRON

Compounds considered biologically active are the bitter principles derived from safranal and the pigments related to the carotenoid crocetin. Among the bitter principles, picrocrocin is the most important. Decomposition of this glucoside by acid hydrolysis gives glucose and the volatile aglycone, safranal (dehydro-β-cyclocitral), and by enzymatic hydrolysis with emulsine gives oxysafranol.

Among the dye materials, the main constituents are the carotenoids crocetin (also called α-crocetin or crocetin-I) and its glycosidic forms digentiobioside (crocin), gentiobioside, glucoside, gentioglucoside and diglucoside; γ-crocetin (monomethyl ester), γ-crocetin (dimethylester), α-carotene, β-carotene, lycopene and zeaxanthin (Corradi and Micheli 1979a; Duquenois 1972; Sampathu *et al.* 1984). Besides the *trans* crocetin isomer, 13-cis-crocetin isomer (Speranza *et al.* 1984) and mangicrocin, α-xanthone-carotenoid glycosidic conjugate (Ghosal *et al.* 1989) has also been isolated. The biological action of these compounds is expressed in various forms including, antidepressant, hypolipidaemic, anti-inflammatory tissue oxygenation, anti-tumour and free radical scavenging activity.

Antidepressant activity

Intraperitoneally applied aqueous and ethanolic extracts of saffron and its constituents safranal and crocin were shown to have antidepressant effects in mice, using the forced swimming test. Immobility time was decreased by saffron extracts in a dose of 200–800 mg/kg, and by safranal (0.15–0.5 mg/kg) and crocin (50–600 mg/kg). Safranal increased swimming time. Climbing time (which is increased by imipramin) is increased by both extracts, safranal (0.5 mg/kg) and crocin (50–600 mg/kg). In the open field test, the ethanolic extract and safranal increased stereotypic behaviour. Safranal and crocin both contribute to the antidepressant effect, with crocin probably acting via re-uptake inhibition of dopamine and norepinephrine, and safranal via serotonin reuptake inhibition (Karimi *et al.* 2001; Hosseinzadeh *et al.* 2004).

Experience from clinical studies is limited to four pilot trials, three of which were designed to demonstrate the antidepressant efficacy of saffron extracts. Daily intake of 100 mg of saffron in milk for 6 weeks led to an improvement of the antioxidative status of patients with coronary heart disease. The susceptibility of lipoproteins to oxidation decreased considerably in the patients (–35.8%) as well as in the healthy volunteers (–42.3%) receiving saffron, whereas no change was found in the controls (increase to 103.6%) (Verma and Bordia 1998). In a clinical double-blind study, 30 patients with mild to moderate depression were either treated with 30 mg of saffron or with 100 mg of imipramine for 6 weeks. The effects were found to be equivalent, with a better tolerability of saffron (Akhondzadeh *et al.* 2004). This pilot study was followed by a placebo-controlled trial in 40 patients suffering from depression. Saffron was found to be significantly superior to placebo (Akhondzadeh *et al.* 2004). In a six-week double-blind pilot study, Noorbala *et al*. (2005) compared the efficacy of an aqueous-ethanolic saffron extract (30 mg/day) with that of fluoxetine (20 mg/day) in 40 outpatients with mild to moderate depression. The equivalence of both treatments was confirmed (Noorbala *et al.* 2005). The trials on depression confirm the observations from the *in vivo* pharmacological studies. However, a full size trial with a design corresponding to the most recent guidelines on depression therapy is needed for a definite conclusion. Still, it is noteworthy that saffron's use against depressive symptoms has a long tradition from antiquity to date. In the countries of origin, saffron tea has the reputation of improving mood – which may well be in line with antidepressant effects. Further research into the use in depression seems worthwhile.

Hypolipidaemic activity

Crocetin has also a hypolipidaemic effect which can be useful in preventing atherosclerosis (Hansel 1977). Intramuscular injection of crocetin into rabbits fed with a cholesterol-containing diet resulted in a large reduction in the severity of atherosclerosis and serum cholesterol levels were reduced by half (Gainer and Chisolm 1974; Gainer and Jones 1975). This finding was considered to be related to the low incidence of cardiovascular disease in the regions of Spain in which saffron was consumed daily (Grisolia 1974). Miller *et al*. (1982) demonstrated that crocetin binds strongly to albumin, and probably to the same albumin binding sites that are occupied by free fatty acids. From their results, they hypothesize that the mechanism by which crocetin reduces the effects of experimental atherosclerosis and increases oxygen diffusivity could be a direct consequence of crocetin binding to the albumin.

Anti-inflammatory activity

Hosseinzadeh and Younesi (2002) tested the anti-inflammatory effects of aqueous and ethanolic extracts from saffron and *Crocus* flowers in mice, using the hot plate and writhing tests. Whereas no effect was found in the hot plate test, the stigmata inhibited the acetic acid induced writhing reflex. The effect could only partially be inhibited by naloxon (Hosseinzadeh and Younesi 2002). The oral application of 125–500 mg/kg of saffron extract to mice had no effect on learning abilities in the passive avoidance test, but distinctly improved the memory of mice pre-damaged with ethanol (Zhang *et al.* 1994). This effect could be attributed to crocin, which does not have an effect in a dose range of 50–200 mg in healthy animals, but in fact improves cognitive functions in animals where memory was artificially impaired application of ethanol (Sugiura *et al.* 1994, 1995).

Tissue oxygenation activity

Various studies with crocetin have also shown the capacity of this carotenoid to promote the diffusivity of oxygen in different tissues. In different *in vitro* tests, crocetin increased the diffusion speed of oxygen through plasma, and provided a net increase in the oxygen available to the capillary endothelial cells in dogs with experimental spinal cord injury (Gainer 1977), in cats with experimental cryogenic brain lesions (Gainer and Nugent 1976), in emphysemic rats (DiLuccio and Gainer 1980) and in rats with haemorrhagic shock (Gainer *et al.* 1993). The *in vitro* experiments also verified the enhanced diffusion of oxygen through plasma due to crocetin (DiLuccio and Gainer 1980). Seyde *et al.* (1986) studied the effects of crocetin on the cerebral cortex of rats subjected to haemorrhage, and the results obtained suggested that this compound improves tissue oxygenation. Two years later, Holloway and Gainer (1988) studied the role of crocetin in the oxygenation in rabbits anaesthesized with urethane and subjected to mild hypoxia induced by a reduction in the minute volume. The increased oxygen pressure in the arterial blood was attributed to increased diffusion through plasma. The rats' blood flow remained constant and was unaffected by crocetin. This compound increased oxygen diffusivity in rats bled 40%, and resulted in increased whole-body oxygen consumption and survival rates. The oxygen uptake by muscle was also increased by oral administration of crocetin (Gainer *et al.* 1993).

Nervo-stimulation activity

Independent of its use as a dye and scent, saffron is also of interest because of its vitamin content; with a content of about 100 μg riboflavin/g it constitutes a good source of this vitamin. In studies with rats, 150 mg of saffron had a beneficial effect equivalent to that of 40 mg pure riboflavin (Rajagopalan *et al.* 1960). These authors also consider the volatile oil constituents responsible for the effect of saffron on central nervous system stimulation. However, it seems that this activity depends on the dose, since other authors report that saffron could cause sedation and even hypnosis at high doses (Benigni *et al.* 1964). Oral administration of saffron may be useful as treatment for neurodegenerative disorders and related memory impairment (Sugiura *et al.* 1995; Abe and Saito 2000).

Pitsikas and Sakellaridis (2006) investigated the effects of extracts of *C. sativus*, on memory in the rat by using the object recognition and the step-through passive avoidance task. In the first study, post-training administration of *Crocus* extracts (30 and 60 g/kg) successfully counteracted extinction of recognition memory in the normal rat, suggesting that *Crocus* extracts modulated storage and/or retrieval of information. In the subsequent study, pre-training treatment with *Crocus* extracts (30 and 60 mg/kg) significantly antagonized the scopolamine (0.75 mg/kg)-induced performance deficits in the step-through passive avoidance test. These results showed that low doses of crocins antagonized extinction of recognition memory in the object recognition test and scopolamine-induced performance deficits in the passive avoidance task. Pitsikas *et al.* (2007) investigated the effects of crocins on recognition and spatial memory, in the rat. For this aim, the object recognition task which evaluated non-spatial working memory and a novel version of the radial water maze which assessed spatial reference and spatial working memory were chosen. In the first study, crocins (15 and 30 mg/kg) counteracted delay-dependent recognition memory deficits in the normal rat, suggesting that these carotenoids modulated storage and/or retrieval of information. In the subsequent study, treatment with crocins (30 mg/kg and to a lesser extent also 15 mg/kg) attenuated scopolamine (0.2 mg/kg)-induced performance deficits in the radial water maze test. These results supported and extended the enhancing effects of crocins on memory and, demonstrated its implication in the mechanisms underlying recognition and spatial memory.

Pitsikas *et al.* (2008) in continuation of their previous studies found that crocins possessed anxiolytic properties. They used the light/dark test and they found that either crocins, at a dose which did not influence animals' motor activity (50 mg/kg), or diazepam (1.5 mg/kg), significantly increased the latency to enter the dark compartment and prolonged the times pent in the lit chamber in the rats. Conversely, lower doses of crocins (15–30 mg/kg) did not substantially modify animals' behaviour. These results indicated that treatment with the active constituents of *C. sativus* induced anxiolytic-like effects in the rat.

Hosseinzadeh *et al.* (2008) evaluated the aphrodisiac activities of *C. sativus* stigma aqueous extract and its constituents, safranal and crocin, in male rats. The aqueous extract (80, 160 and 320 mg/kg body wt.), crocin (100, 200 and 400 mg/kg body wt.), safranal (0.1, 0.2 and 0.4 ml/kg), sildenafil (60 mg/kg body wt., as a positive control) and saline were administered intraperitoneally to male rats. Mounting frequency (MF), intromission frequency (IF), erection frequency (EF), mount latency (ML), intromission latency (IL) and ejaculation latency (EL) were the factors evaluated during the sexual behavior study. Crocin, at all doses, and the extract, especially at doses 160 and 320 mg/kg body wt., increased MF, IF and EF behaviors and reduced EL, IL and ML parameters. Safranal did not show aphrodisiac effects. This study revealed that saffron aqueous extract and its main constituent crocin improved aphrodisiac activity of male rats.

Anti-tumour activity

Anti-tumour activity has been evidenced in various cellular models, and has been extensively reviewed (Wintherhalter and Straubinger 2000; Abdullaev 2004; Abdullaev and Espinosa-Aguirre 2004). The viability of healthy cells regularly remained unaffected, whereas saffron had selective cytotoxic effects on malignous cells, including human cancer cell lines, with effective doses in the low micromolar range.

Orally and topically applied saffron extracts reduced the incidence of artificially induced cancer *in vivo*, inhibited tumour growth rates and prolonged the life span of the test animals (Nair *et al.* 1991a; Das *et al.* 2004). Isolated crocetin protected rat liver cells from the damaging impact of aflatoxins (Wang *et al.* 1991a). At the same time saffron was essentially non-toxic in mice, with an LD 50 of 600 mg extract/kg body weight (Nair *et al.* 1991b). A recently found affinity of saffron extract and the isolated crocins to the sigma-1 receptor (IC 50 30 μM) may explain some of the described anti-tumour effects (Hensel *et al.* 2006).

Nair *et al.* (1991a) studied the antitumour activity of saffron extract against intraperitoneally transplanted sarcoma-180, Ehrlich ascites carcinoma and Dalton's lymphoma ascites tumours in mice. A delay in the onset of tumour formation and an increase in the life span of treated mice compared with the untreated controls were observed. However, *in vitro* studies with the same cell lines indicated that saffron has a cytotoxic effect, and the three kinds of tumour cells were sensitive to the extract at low concentrations, while normal mouse spleen cells were insensitive to it. Pharmacological and biochemical studies showed the complete absence of any toxicological manifestations in the liver, kidney or bladder. Moreover, Nair *et al.* (1991b) found that administration of *C. sativus* extract prolonged the life span of cisplatin-treated mice almost three-fold, without renal toxicity (Boroushaki *et al.* 2008).

Among the active compounds isolated from saffron, crocetin has been most studied for its antitumour activity. Crocetin has decreased the number of tumours and delayed their onset in two different types of tests involving animal tumours (Gainer *et al.* 1976). Mathews (1982) examined the effect of this compound on experimental skin tumours in hairless mice and it seemed to have a small inhibitory effect on the development of skin tumours induced by the application of 9,10-dimethyl-l,2-benzanthracene and croton oil. However, no definitive proof of the prevention of tumours induced by UV-B radiation was obtained.

Investigation of the effect of crocetin on the irradiation of Walker 256 *in vitro* and *in vivo* showed that the carotenoid increased the relative growth of tumour cells and their radiosensitivity (Wilkins and Gainer 1979). In other experiments, crocetin increased the relative growth of normal rat muscle-derived cells *in vitro,* probably by increasing the oxygen transport in the ribosomal-microsomal fraction (Wilkins *et al.* 1977). This supports the hypothesis that crocetin affects cell division enzymatic processes (Wilkins and Wilkins 1979). Abdullaev (1994) studied the effect of crocetin on three malignant human cell lines (HeLa, A549 and VA13). Incubation of these cells with crocetin for 3 h caused a dose-dependent inhibition of nucleic acid and protein synthesis. Crocetin also had a dose-dependent inhibitory effect on DNA and RNA synthesis in isolated nuclei and suppressed the activity of purified RNA polymerase II.

Antistress activity

Saffron is recognized in Ayurvedic therapy as a promoter of non-specific immunological defence. Saffron extracts exhibit significant anti-stress and anti-anxiety activities in animals and humans. In 1989, Ghosal *et al*. isolated an antistress factor from saffron. The novel xanthone-carotenoid glycidic conjugate, mangicrocin, was administered orally (5-25 mg/kg) for five consecutive days and showed significant antistress activity on different tests. The experiment demonstrated that the active part of the molecule is the xanthone moiety (mangiferin), while crocetin (carotenoid moiety) did not elicit any antistress activity.

Free radical scavenging activity

Among the most significant activities of crocin and crocetin are their free radical scavenger properties (Bors *et al.* 1981, 1982; Erben-Russ *et al.* 1987). These carotenoids are used in sperm cryo-conservation for their superoxide scavenger capacity (Paramonova *et al.* 1989). The stabilizing effect of carotenoids on the preservation of sperm is associated with the interaction of carotenoids with oxygen radicals rather than singlet O_2; free radicals probably form a complex with carotenoids, which changes its oxidative capacity.

These experimental data might help to explain the hepatoprotective and anticancer properties demonstrated by other authors (Wang *et al.* 1991a, 1991b). Studies on the hepatoprotective effects of crocin demonstrated its great protective effect in rats treated with aflatoxine B, and dimethylnitrosamine (Lin and Wang 1986). Crocin dyes did not affect hepatic function when they were administered orally to rats at a daily dose of 50 mg/kg for 8 days. A higher dosage of 100 mg/kg for 2 weeks was able to induce hepatic damage, but this effect was completely reversible (Wang *et al.* 1984). After pre-treatment of rats with 2 or 6 mg/kg of crocetin daily for three consecutive days, the hepatic damage produced by aflatoxin B, was significantly suppressed. Crocetin probably has chemopreventive effects on the early acute damage induced by the toxic (Wang *et al.* 1991a). The authors suggest that the protective effect might be due to the hepatic tissues defence mechanisms, which elevate the cytosol glutathiones and the activities of glutathione *S*-transferase and glutathione peroxidase.

Saffron extracts presented also protective effect on renal ischemia-reperfusion-induced oxidative damage in rats (Hosseinzadeh *et al.* 2005), in cerebral ischemia in rats (Hosseinzadeh *et al.* 2005; Saleem *et al.* 2006) in rat hippocampus (Hosseinzadeh and Sadeghnia 2005) and in rat skeletal muscle (Hosseinzadeh *et al.* 2007). The significant radical scavenging activity of crocus has been recently confirmed (Assimopoulou *et al.* 2005; Chen *et al.* 2008; Termentzi and Kokkalou 2008), whereas the significant protection on genotoxic-induced oxidative stress in Swiss albino mice (Premkumar *et al*. 2003) and the amelioration of the oxidative damage in rat brain (Del Angel *et al.* 2007) and other mice organ (Hosseinzadeh *et al.* 2008) have been established.

SAFETY ASPECTS OF SAFFRON

The toxicity of saffron has been found to be quite low. One case of an anaphylactic reaction to saffron has been reported (Wóthrich *et al.* 1997). Lucas *et al*. (2001) assessed the allergenic risk of saffron as very low. In view of the annual production of > 200 tons of saffron, allergic reactions to saffron can be expected to occur in only very rare cases (Lucas *et al.* 2001), which has also been confirmed by the analysis of 589 prick tests on allergies against various spices (Moneret-Vautrin *et al.* 2002).

Oral administration of saffron extract at concentrations from 0.1 to 5 g/kg was non-toxic in mice (Abdullaev 2002). Daily doses of up to 1.5 g of saffron are thought to be safe. Since the dose shown to be efficacious in depression trials corresponded to approximately 30 mg of saffron, there is a large safety margin. Toxic effects are reported with 5 g and above, with a lethal dose of approximately 20 g. Reportedly, saffron has been used for the induction of abortion in doses > 10 g. This dose is said to cause vomiting, uterus bleeding, haematuria, bleedings of the gastrointestinal mucosa as well as vertigo and dizziness. The coloured constituents may accumulate in sclera, skin or mucosa, and may thus mimic icteric complaints (Hensel and Rosing 2003). Usually saffron is recognized as a safe natural spice.

USE OF SAFFRON AS A FEED ADDITIVE

Lipid and protein oxidation of foods has been of great interest because it implies deterioration of colour, flavour, texture and nutritional value (Halliwell and Gutteridge 1999). The oxidative stability of shell eggs in refrigerated storage has not been a major problem because eggs contain compounds that can protect the in-shell system from oxidation. Phosvitin, a yolk protein, and conalbumin, an albumen protein, have both been shown to exert antioxidant activity by inhibiting Fe^{2+} and Cu^{2+} catalyzed reactions, whereas other naturally occurring egg yolk constituents including *α*–tocopherol, xanthophylls and lecithin, have been shown to be effective in preventing lipid oxidation (Cuppert 2001).

Although shell eggs are stable against oxidation, processed eggs can be readily oxidized during refrigerated storage. In the last few years, the susceptibility of processed eggs to lipid oxidation has been of increasing concern due to marketing of dietary "modified" eggs that contain higher levels of ω-3 fatty acids. These highly unsaturated eggs present higher susceptibility to oxidation during storage or processing, particularly, at low pH (Pike and Peng 1988; Botsoglou *et al.* 1998).

Direct use of synthetic antioxidants such as hydroxyanisole, butylated hydroxytoluene, or *tert*-butylated hydroxylquinone, for increasing the oxidative stability of foods is currently approved, but consumer concern over their use (Imaida *et al.* 1983; Okada *et al.* 1990; Botterweck *et al.* 2000) has created a need and prompted research for alternative antioxidants. Thus, the poultry industry would greatly appreciate natural antioxidants that could replace synthetic ones and satisfy consumer demands for production of eggs and meat without residues from substances that have the potential to harm human health.

In the last few years, several studies have suggested dietary supplementation as a very effective means for improving the oxidative stability of eggs and meat. Increasing the level of dietary *α*–tocopherol in poultry feeds the oxidative stability of processed eggs and meat during refrigerated storage has greatly increased (Wahle *et al.* 1993; Cherian *et al.* 1996; Qi and Sim 1998). Besides, feed supplementation with various herbs such as rosemary (Galobart *et al.* 2001) and, particularly, oregano and thyme (Botsoglou *et al.* 1997) extracts has also been shown very promising for improving the oxidative stability of egg yolk and meat.

In a recent study assessing the antioxidant properties of some spices frequently used in the Mediterranean diet, saffron was found to be an effective scavenger of peroxyl radicals in an ox brain phospholipid system compared with rosemary, oregano, annatto, paprika, cumin and common food additives including butylated hydroxyanisole, butylated hydroxytoluene and propyl gallate (Martinez-Tomé *et al.* 2001). Both crocins and crocetins have exhibited substantial antioxidative activity; however, their antioxidative mechanism has not been yet adequately elucidated (Matheson and Rodgers 1982; Ríos *et al.* 1996; Pham *et al.* 2000). Possible incorporation of the antioxidant constituents of saffron into egg yolk and meat through addition of saffron to feeds might help in reducing or eliminating the need for additional oxidative stabilization.

One doubt to the use of saffron as a feed additive could be the fact that saffron is the most expensive cultivated spice, although, it is cultivated on an industrial scale in various countries, including temperate ones. However, it must be noted that at least 30% of saffron samples do not fulfil quality specifications or are considered as waste products (Hensel *et al.* 2006). These saffron by products that have the same composition as the rest of the spice, are discarded for aesthetic reasons and marketed as a relatively cheap by-product of this industrial production. The low cost of this material encouraged its investigation to be used as a promising and sustainable feed additive for his antioxidant and colouring properties, as well as the health promoting ones.

In the literature, little information on the use of dietary saffron as a feed additive is available and is limited to layers and broiler chickens (Botsoglou *et al*. 2005a; Florou-Paneri *et al*. 2006). Botsoglou *et al*. (2005a) investigated the effect of feed supplementation with red stigmas of Greek saffron on the oxidative stability of shell eggs and liquid yolks during refrigerated storage. In this study, laying hens were given feeds supplemented with 10 or 20 mg saffron or 200 mg α-tocopheryl acetate/kg feed. Following 6 weeks feeding, eggs were collected and the rate of lipid oxidation was determined in shell eggs refrigerated stored for two months. Results showed that the extent of lipid oxidation in shell eggs, as measured by malondialdehyde (MDA) formation (Botsoglou *et al*. 1994), differed significantly between the dietary treatments, but did not change with the storage time. Possible transfer of the antioxidant constituents of saffron into the eggs through feeding could inhibit the chain reaction involved in oxidation of the consumed lipids, thus decreasing the oxidation products transferred into yolk. Pham *et al.* (2000) have shown that crocins, the main biologically active constituents of saffron, could prevent the oxidation of linoleic acid *in vitro* as their antioxidative activity was found to be comparable to that of butylated hydroxy anisole.

Since shell eggs were inherently resistant to oxidative deterioration upon refrigerated storage, the same authors (Botsoglou *et al*. 2005a) performed additional experiments in order to evaluate the oxidative stability of the yolk lipids under conditions that could promote lipid oxidation. Thus, the effect of dietary treatments on the oxidative stability of yolks adjusted at pH of 6.2 or 4.2 and agitated for 20 days in presence of light was investigated. Results showed that the extent of lipid oxidation, as measured by MDA formation, differed between the dietary treatments at all time points. Dietary treatments exhibited progressively higher MDA values compared to day 0, however, the rate of lipid oxidation was rather low by day 12 of agitation and it was more intense thereafter. Control yolks at pH 6.2 presented mean MDA values that were higher than those of the group fed 10 mg saffron/kg feed, which in turn were higher than those of the group fed 20 mg saffron/kg feed, a finding suggesting that dietary saffron exerted a dose dependent antioxidative activity. The α-tocopherol supplemented group presented MDA values that were lower compared to all other treatments at all time points. The oxidation profile of yolks adjusted at pH 4.2 showed a similar pattern, however, the rate of oxidation was more intense. Considering that egg yolks from the saffron groups exhibited increased resistance to lipid oxidation compared to the control, it could be assumed that components from saffron passed through feeding into egg yolks providing antioxidant properties.

In another study, Botsoglou *et al*. (2005b) investigated the effect of feeding rosemary, oregano, saffron or α-tocopheryl acetate on hen performance and egg quality. In this study, the experimental feeds contained 200 mg α-tocopheryl acetate, or 5 g rosemary, or 5 g oregano, or 20 mg saffron/kg. At the end of the feeding trial that lasted 56 days, hen performance and some egg quality characteristics were determined, whereas the oxidative stability of the refrigerated stored shell eggs and liquid yolks were also examined. Results showed no significant differences in egg production, feed intake, feed conversion ratio, egg weight and shape, yolk shape, haugh units and shell thickness among treatments. However, yolk colour was significantly improved in the saffron group compared to other groups. The extent of lipid oxidation in shell eggs differed among the dietary treatments, but did not change with storage time. In liquid yolk at pH 6.2, lipid oxidation was higher in the control group compared to other groups. The oregano group presented lower oxidation rate than the rosemary group, but higher than the saffron group, which in turn exhibited higher oxidation rate than the tocopherol group. When liquid yolk was acidified at pH 4.2, the lipid oxidation profile remained unchanged but the rate of lipid oxidation was much more intense.

Florou-Paneri *et al.* (2006) investigated the effect of feed supplementation with red stigmas of Greek saffron on the oxidative stability of breast and thigh muscle tissues. In this study, the chickens were given feeds supplemented with 10 or 20 mg saffron or 200 mg α-tocopheryl acetate/kg feed. Following 42 days feeding, birds were slaughtered, and the oxidative stability of the collected breast and thigh muscle tissue samples were assessed by monitoring MDA formation during refrigerated storage at 4°C for 9 days. The results showed that refrigeration of both raw and cooked tissue samples led to spontaneous increase in the MDA content in all groups. This increase in the MDA content was much more intense in the cooked samples compared to the raw ones. Tissue samples from birds fed the feed supplemented with 200 mg/kg α-tocopheryl acetate showed lowest mean levels of MDA during the 9-day period of refrigerated storage compared to the other groups. The incorporation of saffron in feeds led to modest decrease in the formation of MDA in the samples compared to the control. Saffron supplementation at the level of 20 mg/kg feed was more effective in retarding lipid oxidation in both raw and cooked samples compared to the level of 10 mg saffron /kg feed. Thigh muscle was more susceptible to oxidation than breast muscle in all groups.

CONCLUSIONS

Saffron is the most precious spice in the world. Saffron was not always primarily a spice, but rather a highly valued medicinal plant, for which many uses have been reported. Modern pharmacological research and clinical testing confirmed large parts of traditional knowledge regarding the medicinal effects of saffron. Several biological properties are of interest for future applications of saffron and its major constituents either in veterinary or human medicine. In reference to these properties considered above, we suggest a positive reappraisal of the uses of saffron as an important phytotherapeutic drug. As a feed additive, saffron could be used as a natural colorant and antioxidant promoter since there has been evidence that it has the ability to improve the oxidative stability of poultry meat and eggs. Saffron by-products could serve as the raw material for the preparation of this feed additive.

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