












Journal of Experimental Biology and Agricultural Sciences

<http://www.jebas.org>

ISSN No. 2320 – 8694

Potential benefits of *Glycyrrhiza glabra* (Liquorice) herb, its chemical make-up and significance in safeguarding poultry health: Current scientific knowledge

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Received – May 25, 2023; Revision – June 28, 2023; Accepted – June 30, 2023

Available Online – June 30, 2023

DOI: [http://dx.doi.org/10.18006/2023.11\(3\).462.478](http://dx.doi.org/10.18006/2023.11(3).462.478)

KEYWORDS

Glycyrrhiza glabra

Liquorice

Therapeutic benefits

Pharmaceutical

ABSTRACT

Positive results have been seen when bioactive components from herbal plants are added to poultry diets. Efficacy in feeding, digestion of nutrients, antioxidant health, immunological indices, and other factors can all be improved with the help of these additives, which in turn increases growth rates and improves poultry welfare. Several researchers have used sophisticated herbal formulae that included *Glycyrrhiza glabra* (Liquorice) as an ingredient. Epidemic illnesses, mainly in the respiratory, digestive, and immunological systems, pose the greatest threat to the poultry business. Flavonoids and glycyrrhizin are two of the bioactive compounds in Liquorice. The roots of this plant contain glycyrrhizin at concentrations of 1-9%, which has numerous pharmacological benefits, including anti-infectious, antioxidant, antiviral,

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Peer review under responsibility of Journal of Experimental Biology and Agricultural Sciences.

Production and Hosting by Horizon Publisher India [HPI]
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Poultry

Health

and anti-inflammatory properties. Liquorice extracts are helpful in the treatment of multiple common illnesses. These include problems with the liver, the lungs, and the immunological system. Adding Liquorice to chicken diets improves their productivity in several ways, including fostering organ growth and stimulating digestion and appetite. Liquorice has many beneficial effects on birds, including helping them grow larger bodies, cleansing their systems, and protecting them from free radicals, bacteria, and inflammation. In this article, we'll look at the chemical make-up of liquorice herb, its role in protecting poultry health, and its recent applications and benefits.

1 Introduction

In developing countries like India, the majority of people make their living in agriculture and related industries. Although the agricultural sector's share of India's economy is shrinking, it disproportionately impacts the country's workforce disproportionately. As a result of rising costs and uneconomical holdings, farming in the country has become unprofitable, notwithstanding the country's impressive achievements in food production (Chandran 2021; Buttar et al. 2022; Chandran et al. 2022; Mitra et al. 2022; Rajan et al. 2023). Farmers must therefore rely on unrelated industries such as animal husbandry, dairy farming, poultry industry, and milk manufacturing. Due to its low investment cost and short gestation period, poultry farming has emerged as a key industry in India, with the potential to spur rapid economic growth and assist people experiencing poverty. Backyard poultry also helps the poorest of the poor generate more revenue and feed their families (Kaur et al. 2009; Sheikh et al. 2018; Chandran 2021; Egorov et al. 2021; Prakash et al. 2022; Rajan et al. 2023).

Plants and medicinal herbs possess several useful potentials in protecting the health of humans, animals and poultry and increasing production in animals and poultry (Hartady et al. 2021; El-Sabrou et al. 2023; Zebeaman et al. 2023). These are a rich source of essential nutrients and play critical roles as immunomodulators, antioxidants, antimicrobials, and also as alternative and supportive (adjunctive) to antibiotics under the present evolving menace of emerging antimicrobial drug resistance (Dhama et al. 2014; Dhama et al. 2015b; Yadav et al., 2016; Dhama et al. 2018; Tiwari et al. 2018; Kuralkar and Kuralkar 2021; Uddin et al. 2021; Parham et al. 2020). Medicinal plants have been traditionally used for many years to treat and prevent illness in animals and poultry and have shown promising beneficial, scientifically evidenced impacts in recent times (Verma and Singh 2008; Abdelli et al. 2021; Dhama et al. 2015a; Kuralkar and Kuralkar 2021; Chandran et al. 2022; Seidavi et al. 2022; El-Sabrou et al. 2023). Phytobiotics have been shown to improve growth, gastrointestinal health, antioxidant action, nutrient absorption, and immunity and decrease diarrheal syndrome in poultry (Prakash et al. 2021a; Rafiq et al. 2022). Herbal remedies are frequently employed in the industry. In particular, small- and medium-scale farmers often rely on traditional treatments derived

from plants rather than synthetic pharmaceuticals. Alkaloids, saponins, flavonoids, phenols, and tannins are only a few examples of the phytochemicals found in medicinal plants that give them therapeutic properties and make them inexpensive. Despite the advances in modern medicine, many Indian farmers still rely on traditional herbal treatments for poultry health (Egrov et al. 2021; Prakash et al. 2021a; Prakash et al. 2021b; Kumar et al. 2022; Kumari et al. 2022; Rajan et al. 2023).

Glycyrrhiza glabra Linn (Liquorice or licorice) is one of the most used herbs in Ayurvedic medicine (Jatav et al. 2011). It is also most often traditionally used medicine in Europe and China for long before (Zhou et al. 2019). *Glycyrrhiza glabra*, commonly called Liquorice or sweetwood or Mulaithi, is a medicine and a flavouring agent. The host's metabolism and immune system are improved using Liquorice, mainly used to treat oral, gastrointestinal, and liver problems. The blood testosterone level of women is lowered by its use, and aplastic anemia is helped by it. It is also a herbal medicine for gastritis and respiratory tract infections. Immunodeficiency illnesses, such as acute immunodeficiency syndrome, are also treatable with liquorice extract, which has been used for years to treat autoimmune conditions (Kaur et al. 2013; Sharma and Agrawal 2013). There are both estrogenic and anti-estrogenic properties in licorice root. According to research conducted by Kaur et al. (2013), this plant is also effective in treating hormone-related issues in females.

Glycyrrhiza, the genus from which Liquorice comes, is a family of roughly fourteen species. The roots of this plant have been used medicinally for more than 4000 years. As Ajaib et al. (2013) reported, *G. glabra* is a plant whose roots treat genito-urinary conditions due to their tonic, demulcent, laxative, and emollient properties. Roots of the *glycyrrhiza* plant are used to alleviate a cough due to their demulcent and expectorant qualities. Liquorice roots, when chewed fresh, have multiple uses as a breath mint which helps in teething, and make whiten teeth. Liquorice root is a popular supplement for treating gastrointestinal issues, menopausal symptoms, and bacterial infections in modern times. Liquorice tea is also used to cure a sore throat, and topical gels help with skin issues such as acne and eczema. Liquorice leaves when used fresh, work as a natural antiseptic for cuts and scrapes. It is also used to deal with conditions like diabetes, stomach ulcers, Addison's

disease, renal issues, and infections caused by bacteria and viruses. Increasing oxygen and nutrition delivery to the scalp helps hair grow from its base. Diarrhea, fever, fever with delirium, and anuria are all conditions that benefit from their inclusion in therapeutic oils. Liquorice powder can also be found in desserts, ice creams, baked products, and soft drinks (Bala et al. 2021).

Numerous pharmacological benefits, including immunomodulatory, antioxidant, antiviral, and anti-inflammatory, have been ascribed to various bioactive components of Liquorice, such as glycyrrhizin and flavonoids (Farak et al. 2012). Liquorice, when added to poultry diets, promotes healthy organ development, boosting growth and productivity. Quails fed a diet containing 200 ppm of liquorice root extract and 1% probiotic gained more weight and muscle and consumed more feed than control quails (Gowthaman et al. 2021). Broiler hens with 2 g/kg of Liquorice in their feed or 0.3 g/l of licorice in their water had lower levels of abdominal fat. Alagawany et al. (2019a, b) found that laying hen performance improved when Liquorice was added to chicken diets at concentrations up to 0.5% before sexual maturity. Poultry birds fed Liquorice in drinking water at 1, 2, or 4 mg/kg body weight saw an improvement in the flavor and aroma of their carcasses. The present review paper tried to outline the morphology of the plant, the chemical make-up of Liquorice, and the therapeutic and pharmacological effects of Liquorice on poultry.

2 Characterization of plant

Glycyrrhiza glabra L. (Figure 1) belongs to the Fabaceae family (Sheidai et al. 2008). Nearly 30 different species of *Glycyrrhiza*

have been identified, and even within the *G. glabra* species, there are many different cultivars. Some examples include *G. glabravar typica*, which grows in horizontal stolons and has brown bark, similar characteristics have been reported for *G. glabra glandulifera* and *G. glabravar violaceae* (Nomura et al. 2002; Martins et al. 2016). *G. glabra* is a perennial herb that can reach a height of 2.5 meters. Pinnately complex leaves accompany the purple or white flowers. The fruit is oblong, and it's filled with seeds. About 1.5cm in length, the taproot branches out into smaller, 1.25cm long roots, from which the woody stolons emerge. Together with the root, they make up commercial Liquorice and can grow to 8 meters when dried and cut. Root fragments break apart fibrously, exposing a yellowish core with a distinct odor and flavor. The flower spikes on this plant resemble peas and are around 10 to 15 centimetres long. You can find purple, white, and yellow spikes. Narrow, papilionaceous flowers in shades of lavender and violet bloom on spikes in the plant's axils. The calyx is covered in glandular hairs in short and campanulate, with lanceolate apexes (Kaur et al. 2013).

Pinnately complex leaves with 9–17 oblong to lanceolate leaflets arranged in pairs on the stem. The flowers are tiny and range in hue from purple to bluish-white, while the pods are flat and oblong to linear. The seeds are tiny and dark in color, with a diameter of around 2.5 mm and a weight of 6.2 g per thousand seeds. Up to 1.5 cm in length, the pod-like fruit is erect, glabrous, and pitted reticulately with 3–5 brown reniform seeds (Kaur et al. 2013). Using nitrogen-fixing bacteria in association with *G. glabra*, nitrogen may be extracted from the air. There is



Figure 1 *Glycyrrhiza glabra* (Liquorice)

evidence that certain Mesorhizobium strains cause nodules on *G. glabra* and other *Glycyrrhiza* species. *Agrobacterium*, *Sinorhizobium*, *Rhizobium*, and *Paenibacillus* bacteria have also been found on rare occasions, although these bacteria are either not very effective at fixing nitrogen or are quite rare (Montonen et al. 2012).

3 Chemical composition and bioactive compounds of Liquorice

Liquorice has a wide variety of compounds like phytosterols, starch, sugars, amino acids, tannins, coumarins, choline, and vitamins (such as ascorbic acid/ vitamin C) (Alagawany et al. 2019a; Alagawany et al. 2019b). Liquorice root contains mainly glycyrrhizin as an active ingredient. The glycyrrhizinic acid content of a plant is highest in the primary root, then in the lateral

roots, and finally in the above-ground sections of the plant, which are typically thrown away as garbage (Farag et al. 1984). An extract of liquorice root may contain as much as 25 percent glycyrrhizin. Magnesium, potassium, and calcium salts are also reported to be abundant in this substance, consisting of one molecule of glycyrrhetic acid and two molecules of glucuronic acid. Since glycyrrhizin in liquorice extract (LE) is around 50 times sweeter than sucrose, it has been used instead of sucrose (Hayashi et al. 2009). Raw Liquorice comprises carbohydrates, fiber, protein, silica, and very little fat. In addition, liquorice root ash and moisture levels were measured at 7.70% and 6.80%, respectively. In addition, LE had a calcium level of 1720 mg/100 g and phosphorus content of 78 mg/100 g; its main amino acid components were proline, aspartate, alanine, and glutamic acid (Omer et al. 2014).

Table 1 Liquorice's bioactive constituents are responsible for many of its useful characteristics

S. No	Functional property	Bioactive constituents
1	Antioxidant activity	Licochalone, Glabridin, Isoliquiritigenin, Licocoumarin
2	Anti-HIV	Glycyrrhizin
3	Anticancer activity	Glycyrrhetic acid, Glycyrrhizin
4	Antitussive activity	Glycyrrhizin
5	Antiulcer activity	Glabridin, glabrene, glycyrrhizinic acid
6	Analgesic and uterine relaxant	Isoliquiritigenin
7	Antihyper glycemc activity	18-β-glycyrrhetic acid, glycyrrhizin
8	Memory enhancer	Glabridin
9	Antimycobacterial activity	Glabridin
10	Antiviral activity	Glycyrrhizin, licochalcones, glycyrrhetic acid
11	Corticosteroid activity	18-β-glycyrrhetic acid
12	Hepatocellular carcinoma	Glycyrrhizin
13	Antithrombin activity	Glycyrrhizin, isoliquiritin
14	Estrogenic activity	Glabrene, liquiritigenin
15	Antimycobacterial activity	Glabrene, liquiritigenin
16	Chronic hepatitis C	Glycyrrhizin
17	Spasmolytic	Liquirtin
18	Muscle relaxant	Rhamnoglucoside
19	Hepatoprotective activity	Glycyrrhizin
20	Anti-allergic activity	8-β-glycyrrhetic acid, liquiritigenin, glycyrrhizin
21	Immunostimulatory activity	Glycyrrhetic acid
22	Anti-inflammatory activity	Liquiritoside, Lichoalcone A, glycyrrhetic acid
23	Antioxidant activity	Licochalcone, glabridin, licocoumarin

Source: Kaur et al. (2013); Alagawany et al. (2019a,b); Prakash et al. (2021a); Prakash et al. (2021b); Kumari et al. (2022); Reda et al. (2021)

Liquorice has been analyzed and contains bioactive components like saponins, flavonoids, and triterpenes (Farak et al. 2012). Liquorice's distinctively pleasant flavour originates in its saponin content. Because of variations in processing, geographical origin, and harvesting techniques, the concentration of these chemicals can range widely. Liquorice root also contains 49 phenolic compounds and 15 distinct saponins. Liquorice gets its distinctive yellow hue from a variety of flavonoids. Some of the essential flavonoids are the glycoside liquiritigenin (4',7-dihydroxyflavanone) and isoliquiritigenin (2',4,4'-tri-hydroxychalcone) found in liquiriti fruit. Root dry weight typically contains between 0.08 to 0.35% glabridin, the primary isoflavone (Wang et al. 2015; Rizzato et al. 2017). Stilbene derivatives from liquorice leaves and derivatives of caffeic acid esters are two examples of chemicals that have been isolated in trace levels. In addition to fatty acids and phenols (guaiaicol), saturated linear gamma-lactones have been isolated (Martins et al. 2016).

Many people utilize Liquorice for its ethnopharmacological uses. Moreover, the antioxidant and anti-tumour activities of Liquorice and the compounds derived from it have made their use widespread. 2.5% glycyrrhizin is estimated to be included in at least 71% of traditional Japanese Kampo remedies (Hayashi et al.

2009; Leite et al. 2022). Injectable and tablet (Glycyron) formulations of Liquorice are used in Japan as prescription drugs for treating various medical conditions. Many medications contain liquorice extracts, and one derivative of glycyrrhetic acid, 3- β -O-hemisuccinate, is currently on the market for medical usage (Petropoulos et al. 2016). Hepatitis A and C, Human Immunodeficiency Virus, Herpes Zoster, Herpes simplex, and Cytomegalovirus are only some of the RNA and DNA viruses that have been proven to be inhibited by glycyrrhizin and glycyrrhizic acid. Pseudoaldosterone syndrome is brought on by glycyrrhizin and its metabolites, which prevent aldosterone from metabolizing in the liver and reduce 5(beta) reductase activity. This research was conducted by Ajaib et al. (2013). Liquorice's bioactive components help the plant achieve many valuable effects, as in Table 1.

Methods for extracting bioactive compounds from Liquorice include supercritical carbon dioxide-assisted, microwave-assisted, ultrasound-assisted, and multistage counter-current extraction. Several of the proposed procedures employ potentially dangerous solvents in the liquid phase of the extraction process. Extraction yields are increased, and organic solvent usage is decreased when solid-liquid extraction technologies are used as opposed to traditional methods (Karkanis et al. 2018). Increased yields can be

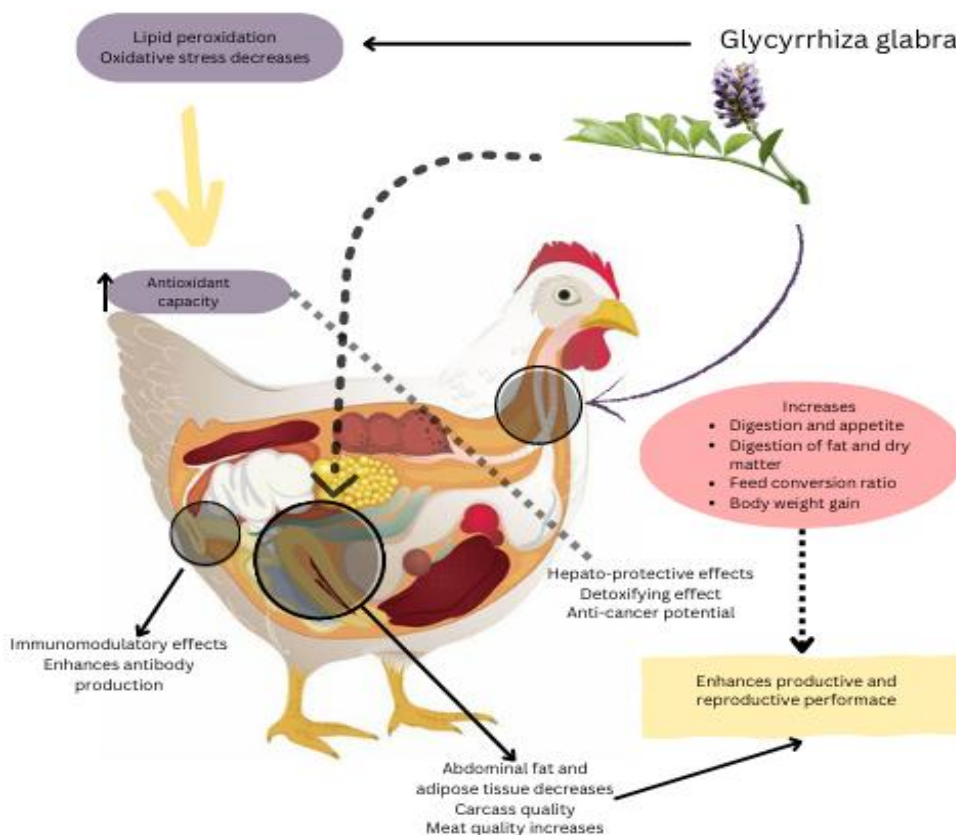


Figure 2 Therapeutic and health benefits of *Glycyrrhiza glabra* supplementation in poultry

achieved in about 4-5 minutes of microwave-aided extraction of roots in an ethanol-water mixture, compared to the typical extraction time of 20-24 hours (Pan et al. 2016). The hazardous effects of chemical solvents can be reduced using supercritical carbon dioxide. More study is needed before the herbal business can use the supercritical extraction process, although it is more efficient and has fewer negative impacts on the environment than conventional methods (Hedayati and Ghoreishi 2016)

4 Beneficial effects of *Glycyrrhiza glabra* on poultry

Glycyrrhiza glabra, commonly known as Licorice, has been valued for centuries due to its medicinal and therapeutic

characteristics (Karkanis et al. 2018). Glycyrrhizin, found in licorice root, is a triterpene saponin a thousand times sweeter than sucrose (Pastorino et al. 2018). The roots contain glycyrrhizin, a pharmacologically active compound with actions as diverse as antioxidant, antiviral, anti-infectious, and anti-inflammatory. Licorice has been used to treat anything from mouth ulcers to arthritis, and it may even have anti-cancer, immune-modulating, antibacterial, detoxifying, and growth-promoting effects. Licorice supplements, when fed to chickens, have been shown to improve productivity by promoting organ growth and stoking the animals' appetites and digestive fires (Alagawany et al. 2019a; Alagawany et al. 2019b). However, it also serves an essential role as a natural anticoccidial agent, with preliminary evidence suggesting that a

Table 2 Poultry specific studies incorporating the inclusion of Licorice in feed

Category of poultry	Plant part and its dosage	Functional property	Results	References
Broilers	Liquorice extract (0.4 g/L in drinking water)	Immunomodulatory effect	Gains in body mass, feed consumption, and feed conversion ratio. Hypolipidemic, hypoglycemic, hepatoprotective, immunostimulant, and antioxidant properties are only few of the many biological activity demonstrated by licorice. The morphometric examination of various intestinal characteristics showed that the group supplemented with Licorice at a dose of 0.4 gm/L had significantly longer and wider intestinal villi and a greater ratio of villi length to crypt depth than the other groups. Licorice 0.4 gm/L enhanced the proportion of CD3+ T cells in the duodenum and ileum, relative to the control group. Superoxide dismutase and catalase mRNA expression, in addition to growth-related gene expression and lipid-metabolism-related gene expression, were all significantly upregulated in the liver in response to licorice supplementation. By influencing the expression of genes involved in growth, lipid metabolism, and antioxidant activity, we found that licorice supplementation improved the productivity of broiler chickens	Abo-Samaha et al. (2022)
Broilers	Liquorice extract (1 g/kg of diet)	Improves gut health and effective against <i>Campylobacter jejuni</i>	Lowered <i>Campylobacter jejuni</i> shedding from sick birds, boosted growth performance, and affected intestinal integrity maintenance.	Ibrahim et al. (2020)
Chicken		Effective against Mycoplasmosis	Reduced inflammation in the trachea and lungs after Mycoplasma infection. Inhibits Mycoplasma-induced inflammation and apoptosis by downregulating virulence genes (MMP2/MMP9) via the JNK and p38 signaling pathways. The findings suggest it has potential as an alternate antibiotic for the prevention of Mycoplasmosis infection.	Wang et al. (2022)
Japanese quails	Liquorice powder (750-1000 mg/kg diet)	Improves the gastrointestinal health	Resulted in significant decreases in total bacteria, coliforms, <i>E. coli</i> , and Salmonella. Benefits include optimizing performance, immunity, and antioxidant capacity while also preserving a balanced gut microbiome.	Reda et al. (2021)
Chickens	Liquorice extract (0.4% in drinking water)	Antioxidant property	Inhibit abdominal fat accumulation	Alagawany et al. (2021)

combination of *G. glabra* and *Echinacea purpurea* could be used to effectively combat coccidiosis in the poultry sector (Ghafouri et al. 2023). The beneficial effects of liquorice supplementation in poultry are depicted in Figure 2. Evidence from chicken-specific studies (Table 2) shows that *G. glabra* could play a significant effect if added to poultry feed.

5 Antimicrobial effect of Liquorice

The most widely studied pharmacological effects of licorice are its antiviral and antibacterial capabilities. Viral and other microbial infections have a substantial impact on a variety of diseases, especially in developing countries. Liquorice deserves more attention for its extraordinary qualities because of the importance of developing safe and effective antiviral or antibacterial medications. More than 20 triterpenoids and about 300 flavonoids can be found in liquorice. These active components are glabridin, glycyrrhizin, liquiritigenin, licochalcone E, licochalcone A, and 18- β -glycyrrhetic acid (Wang et al. 2015).

6 Antibacterial effect

Various *in-vitro* studies have shown that methanolic extracts of Glycyrrhiza roots are significantly more effective than water extracts at inhibiting the growth of various bacteria, including *Bacillus subtilis*, *Pseudomonas syringae* pv. tomato, *Agrobacterium tumefaciens*, and *B. cereus*. In a laboratory setting, it was discovered that Liquorice's ethanolic extract inhibited the growth of three different types of bacteria, including *B. subtilis*, *Staphylococcus aureus* and *Candida albicans* (Gowthaman et al. 2021). Chloroform, ether, and acetone solvents are also used to extract active ingredients of Liquorice, and all these extracts showed antibacterial activity against both gram-positive (*Staphylococcus aureus* and *B. subtilis*) and gram-negative (*P. aeruginosa* and *E. coli*) bacteria (Nitalikar et al. 2010). The glycyrrhizic acid present in Liquorice is effective against *P. aeruginosa* bacteria (Chakotiya et al. 2016). Yeast and mould growth, lactic acid bacteria, and enterococci prevalence were not affected by five weeks of supplementation with different concentrations of Liquorice in Japanese quail. On the other hand, adding Liquorice to the diet drastically decreased the number of bacteria, particularly coliforms. The highest concentrations of the studied microorganisms were found in the control group that was not fed Liquorice (Alagawany et al. 2021).

7 Antiviral effect

There are 73 bioactive components and 91 potential targets from Liquorice's separated components. GL and 18-beta-glycyrrhetic acid (GA) are two triterpenoids that have been the subject of extensive research and have been found to have

antiviral properties. Glycyrrhizin refers to a compound found primarily in liquorice roots. Numerous studies conducted in recent years have demonstrated GL's antiviral effectiveness. Introducing infectious anti-hepatitis C virus (HCV) particles into a cell was the precise point of attack for GL. Glycyrrhizin is also a potent antiviral agent, inhibiting the expression and replication of viruses by decreasing the adhesion force and stress experienced by viruses and decreasing the binding of HMGB1 to DNA. Fewer studies have been conducted on GA's antiviral activity than GL.

18-beta-glycyrrhetic acid treatment, administered after virus entry, inhibited rotavirus replication. After viral adsorption, adding GA to infected cultures resulted in a 99% reduction in rotavirus yields. Both GA and GL have antiviral properties; however, they are different. Human respiratory syncytial virus (HRSV) and rotavirus do not match GA's antiviral properties. The mechanisms by which these chemicals fight viruses, however, are similar. GA prevents viruses from attaching to cells, slows their growth, and stimulates the activity of the host cells.

In vitro research on the herpes simplex virus 1 (HSV-1) has demonstrated that aqueous extracts of liquorice roots have an antiherpetic effect. Possible mechanisms for this antiherpetic effect include *G. glabra*'s role in blocking HSV attachment to host cells when in contact with the extract. *G. glabra* aqueous extract has an antiadhesive activity that prevents HSV-1 from sticking to Vero cells *in vitro*, and the virus can be inactivated. Evidence suggests that pigeons given LE are less likely to spread Paramyxovirus type 1 (Dziewulska et al. 2018). Glycyrrhizin is a powerful immune stimulant, and it has been shown to have significant antiviral effects against duck hepatitis virus (DHV) (Soufy et al. 2012). Newcastle disease virus (NDV) has also been demonstrated to be susceptible to inhibition by *G. glabra* extracts (Omer et al. 2014). Higher antibody titers against NDV and an increased cellular immunological response in broilers treated with glycyrrhizic acid at 60 g/mL drinking water were observed (Ocampo et al. 2016). In an *in vivo* antiviral study, *G. glabra* extract at a concentration of 300 g/mL was found to have significant antiviral efficacy against NDV. Compared to untreated embryonated eggs, those injected with NDV and afterwards treated with the herbal extract had a higher survival rate and no detectable virus in their allantoic fluids (Ashraf et al. 2017). The anti-flu properties of glycyrrhizin have also been demonstrated in laboratory studies. Contact between the virus and the cell membrane is essential for virus entry and results in decreased endocytotic activity and virus uptake (Wolkerstorfer et al. 2009). In addition to its effectiveness against the Hepatitis C virus, glycyrrhizin also has hepatoprotective properties by shielding the liver from oxidative damage.

8 Antioxidant effect of Liquorice

Several mechanisms contribute to licorice's antioxidant and anti-inflammatory effects: the mitochondria are protected from lipid peroxidation, free radicals are scavenged, antioxidant enzymes are activated, and the activity of phospholipase A2, a key player in many inflammatory processes, is suppressed by licorice extracts. Cellular oxidation is slowed by licochalcone A, but liquorice flavonoids trigger inflammation. The flavonoid NF-kB signaling pathway may be targeted by liquiritigenin, glycyrrhizic acid and liquiritin to inhibit the secretion of inflammatory cytokines; these compounds reduce the expression levels of pro-inflammatory cytokines in the liver. Glycyrrhizic acid reduces prostaglandin E2 synthesis and cyclooxygenase activity (Alagawany et al. 2019a, b), and it also has indirect effects on platelet aggregation and inflammatory markers. In addition, it was discovered that glycyrrhetic acid's delayed release of cortisol might cause high oxidation levels and increased cardiac weight in chickens (Awadein et al. 2010).

The antioxidant effects of Liquorice have been studied extensively in the laboratory and on living organisms. Glabridin was determined to be the most powerful antioxidant out of seven substances found to have antioxidant properties against the oxidation of low-density-lipoproteins by Vaya et al. (1997). In a separate investigation, various liquorice species were tested for their ability to scavenge ABTS+ radicals and prevent lipid peroxidation. In particular, lipid peroxidation was dose-dependently correlated with antioxidant activity (Fu et al., 2013; Gowthaman et al. 2021). Antioxidant feed levels for Japanese quails were also monitored in a separate experiment. Malondialdehyde, superoxide dismutase and total antioxidant capacity were shown to be significantly altered upon administration of Liquorice (Alagawany et al. 2021).

9 Immunomodulatory effect of Liquorice

Extracts of Liquorice have a beneficial effect on the immune systems of chickens. They can boost their immune system's response and increase their output with the help of this herb. Antibody titers against nonspecific and specific antigens were induced in the broilers by supplementing their diet with 0.1% liquorice extract. Liquorice root extract was augmented to the commercial broiler chicks to examine if it altered their immune profile. Total serum protein, albumin, globulin, as well as the albumin/globulin (A/G) ratio were estimated from three sets of chicks to characterize their biochemical status. This criterion divided the chicks into three groups: those given 1% of powdered *G. glabra* crude extract powder, those shown 0.1%, and those given no *G. glabra* extract. Measures of humoral immunity included total and differential white blood cell (WBC) counts, hemagglutination (HA) inhibition titre against LaSota strain of

NDV, and HA titre against sheep red blood cells antigens. The results showed that chicks fed a powdered extract of 0.1% liquorice had markedly enhanced immune responses. Feed supplements made from natural ingredients are also used to boost immunity because they increase white blood cell counts and, in consequence, interferon levels. The laying hens' cellular immunity improved after LE (50 g/mL) was added to their diet. Strong immunological activity has been attributed to the glycyrrhiza polysaccharide, which is involved in numerous facets of immune regulation. The ability to phagocytose was also improved in chicken mononuclear cells and granulocytes after exposure to LE (Alagawany et al. 2019a,b).

Adding Liquorice to broilers' diet improved the animals' immunity and general health by increasing the weight of immune organs (Alexyuk et al. 2019). Immunomodulation and the production of interleukins by glycyrrhetic acid promote the development of T cells, antibodies and IFN- γ (gamma interferon), all hinting at the compound's antiviral potential. Feeding broilers, LE did not affect the immune system (Alagawany et al. 2019a; Alagawany et al. 2019b). Liquorice's immunostimulatory, anti-inflammatory, and antimicrobial properties are because of the active components found in the herb. Glycyrrhiza polysaccharides (GPS) enhance both Th1 as well as Th2 responses by promoting IL-1 β ; IL-2; IL-4; and IFN- γ expression while augmenting the proportion of both T helper cells and cytotoxic T cells in the gastrointestinal tract (Wu et al. 2022a). Not only these, but such polysaccharides also show an immune boosting effect in a dose-dependent manner which can augment immunity of the New Castle disease virus vaccine in chickens (Wu et al. 2022b). Ocampo et al. (2016) found that influencing humoral and cell-mediated immune responses can improve poultry immunity, reduce the likelihood of viral infections, and serve as an adjuvant treatment for existing viral disorders. The levels of immunological markers (IgG, IgM, and lysozyme) in Japanese quail that had been fed were measured in an experiment. While plasma lysozyme activity was comparable between groups, both IgM and IgG were significantly modified. These parameters were shown to be elevated after being given Liquorice (Alagawany et al., 2021).

10 Effect of Liquorice on growth performance

Feed intake increased at 21 and 42 days after adding 0.4% LE to the broilers' drinking water, but there was no change in body weight across the ages. It was discovered that feeding broilers an additional 1% LE in their base diet led to more significant gains in body weight and feed efficiency at 42 days compared to the control group. In addition, the output of heat-stressed broiler chickens was enhanced by LE. *G. glabra*-supplemented diet improved their growth performance by fostering organ growth. Additionally, 2.5 g/kg *G. glabra* added to broiler feed improved digestion and appetite. In addition, feeding up to

0.5% *G. glabra* to developing pullets enhanced their performance, leading to healthier, more productive hens later. Compared to untreated controls, broilers fed glycyrrhizic acid (60 g/mL in water) had higher final body weight, better FCR, and lower mortality. The feed consumption of laying hens was not significantly altered by supplementing the standard diet with 0.5, 1.0, 1.5, or 2.0% liquorice powder. Adding 5g of Liquorice per kilogram of broiler feed had no discernible effect on any of the abovementioned metrics, including feed intake, feed conversion rate, body weight, livability index, or productivity index. The addition of 0.1, 0.2, or 0.3 mg LE/L to the drinking water of broiler chicks did not affect the animals' body weight, feed intake, or feed efficiency as compared to the control group (Alagawany et al. 2019a; Alagawany et al. 2019b). It has been found that when the diet of broilers is supplemented with 600mg/kg of Glycyrrhiza polysaccharide, it shows an optimal effect on growth. In the poultry production industry, the polysaccharide can also be used as a feed additive (Zhao et al. 2023).

Japanese quail and broiler chickens have been the primary subjects of studies on the effects of adding liquorice root supplements to their diets, with the greatest benefits seen in the latter when the supplement was fed at a rate of 2.5g/kg. When LE was added to the drinking water of quails at concentrations of 100 mg and 450 mg/L, the birds gained more weight overall and at harvest. However, only male birds displayed these outcomes. Similar weight increases and lower FCR were observed in quails fed a combination of 200ppm LE and 1% probiotic. Feeding broiler chickens a variety of Liquorice and garlic has been proven to improve the birds' performance in terms of growth (Gowthaman et al. 2021). A considerable weight gain was observed in broiler chickens when aqueous LE was added to their drinking water (Beski et al. 2019). This was accompanied by no changes in the chickens' appetite, thirst, or feed consumption. The addition of Liquorice to broiler chickens, on the other hand, resulted in lower rates of feed consumption and lower rates of weight gain compared to the control group. Liquorice's flavonoids may help you lose weight by decreasing your body's appetite and cravings for fatty foods. As the herbal plants in a diet increase, the risk of nutritional imbalances and adverse effects on body metabolism rises. This has been linked to a decline in growth performance.

A study on 40-week-old hundred egg-laying hens found that diets supplemented with LE increased functional egg production and modulated laying hen productivity by decreasing egg cholesterol and low-density lipoprotein (LDL) while increasing high-density lipoprotein (HDL) and total antioxidant capacity in plasma. Liquorice in feed or water has been demonstrated to improve poultry performance, carcass

traits, and meat quality. Broiler chicks exposed to a high stocking density gained weight more quickly when given a probiotic and LE (500 ppm). Adding LE to broiler hens' drinking water is a viable alternative to giving them antibiotics to speed up their growth. The body weight increase of birds raised at high stocking density was stimulated by LE (500 ppm) but not by the feed conversion rate. Probiotics (200 ppm) were added, and the results improved even more.

Most research indicates that administering licorice root extract to broiler chickens has no discernible effect on the birds' body weight, feed consumption, or feed efficiency. Liquorice supplementation had a significant impact on body weight, daily body weight growth, daily feed consumption, and feed conversion rate in 3-week-old Japanese quail, and this effect persisted even after the feeding trial was over (5 weeks). At three weeks of age, the groups supplemented with 750 and 1000 mg/kg of Liquorice had the highest body weight, daily body weight growth and the lowest feed efficiency values. The most outstanding feed intake values were found in the groups that were given meals containing either 0 or 250 mg/kg of Liquorice. After five weeks, the group fed a diet containing 750 mg/kg of Liquorice had the highest body weight and fat-free mass values, while the groups fed diets containing 0 and 250 mg/kg of Liquorice had the lowest feed conversion rate values (Alagawany et al. 2021). Similarly, supplementing broiler chickens' diets with 2 and 3 g/kg of licorice extracts improved their physiological health and growth performance (Toson et al. 2023).

11 Effect of Liquorice on carcass quality and Yield

There is scanty published research on how feeding the licorice plant affects carcasses and meat quality. The hydrophobic flavonoids in the licorice, as reported by Moradi et al. (2017) and confirmed by Gowthaman et al. (2021), were responsible for the significant reduction in abdominal fat in broiler chicken that was supplemented with 0.3g of liquorice/L of water. While adding 0.45g liquorice extract/L water significantly reduced belly fat in broiler chickens, it did not affect the carcass output of birds raised in a heat-stressed environment. Broiler chickens given LE supplementation did not show any discernible changes in the relative weights of their internal organs (Sedghi et al. 2010; Moradi et al. 2014). Similarly, supplementing Japanese quail with 200 ppm, LE had no appreciable effect on the weights of their spleens or gizzards while leading to increased relative liver weights. In one study, supplementing Japanese quail with liquorice root extract considerably boosted the carcass yield percentage (Myandoab and Hosseini 2012); however, in another study, supplementing with LE had no significant potential effect on the carcass yield (Hosny et al. 2020). Beski et al. (2019) found no evidence that increasing

broiler chickens' drinking water with aqueous LE altered their visceral organ weights, carcass cuts, or intestine histomorphology. Liquorice supplementation led to a rise in organ weights (Rezaei et al. 2014). Liquorice supplements dramatically enhanced the bursa and spleen weight of broiler chickens, and immunological organs, according to Kalanter et al. (2017). Liquorice's antioxidant activity, which influences lipid and protein metabolism, has been associated with improved chicken carcass features and meat quality, as reported by Michaelis et al. (2011). Compound herbal additive B (CHAB) containing Liquorice apart from *Atractylodes*, *Codonopsis pilosula* and *Poria cocos* can cause an improvement in the quality of meat in Hungarian white geese (Fu et al. 2023).

12 Effect of Liquorice on blood chemistry

Human and animal studies found that Liquorice is effective in dyslipidemia and hyperglycemia in cases of metabolic syndrome (Jafari et al. 2021). However, broiler chicken and Japanese quails had a dose-dependent increase in plasma glucose when given liquorice root extract or powder in their diets (Al-Daraji 2012; Dogan et al. 2018a; Dogan et al. 2018b). Since the saponin glycoside glycyrrhizin is 60 times sweeter in taste than sucrose (Roshan et al. 2012), it may be responsible for this increase in blood sugar. However, only a few trials in broiler chickens supplemented with LE (Moradi et al. 2017) or *G. glabra* (Rezaei et al. 2014) have indicated a reduction in serum glucose levels. Supplementation with LE did not significantly affect plasma glucose levels in broiler chicken (Sedghi et al. 2010) or Japanese quail (Myandoab and Hosseini 2012; Al-Sofee 2018, Abdul-Majeed 2019). Toson et al. (2023) found that when LE was added to the chickens' diets, the animals had lower levels of plasma triglycerides, cholesterol, urea, uric acid, and total cholesterol while developing more significant levels of antioxidant markers, RBCs, haemoglobin, WBCs, plasma complete protein, and albumin.

13 Effect on total proteins and their fractions

Japanese quail serum TP significantly increased when crushed liquorice root was introduced to their meal (Al-Sofee 2018). *G. glabra* supplementation in broiler chicken diets led to similar results (Rezaei et al. 2014). On the other hand, another study indicated that the serum total protein levels of Japanese quail treated with liquorice root powder were unaffected (Abdul-Majeed 2019). However, a study of broiler chickens at six weeks of age reported no significant changes in serum albumin, globulin, and albumin: globulin ratio (Jagadeeswaran et al. 2012). Blood total protein and globulin levels were highest in the birds given the 750-mg licorice diet, with the A/G ratio being lowest in those given the 250-mg licorice diet (Reda et al. 2021).

14 Effect on blood glucose

The plasma glucose levels of Japanese quails and broiler chickens were found to rise dose-dependent when liquorice root extract or powder was added to their diets (Al-Daraji 2012; Dogan et al. 2018b). The saponin glycoside glycyrrhizin, which is sixty times sweeter than sucrose, may be to blame for this increase in blood sugar (Roshan et al. 2012; Dogan et al. 2018a). However, some studies show that giving LE or *G. glabra* to broiler chicks reduces their blood sugar. The plasma glucose levels of Japanese quail and broiler chickens were not significantly different after receiving LE supplementation (Gowthaman et al. 2021).

15 Effect on heterophils, lymphocytes and heterophil-to-lymphocyte ratio

Heterophils and the H/L ratio were shown to change in response to heat stress in chicken (Yalcin et al. 2003) and Japanese quail (Mahmoud et al. 2013). An accurate measure of heat stress in hens is the H/L ratio (Gowthaman et al. 2021). Adding LE to the water supply for broiler chickens raised in hot conditions has significantly reduced the H/L ratio (Al-Daraji 2012). According to two previous studies, LE did not affect the percentage of lymphocytes, heterophils, monocytes, or the H/L ratio in broiler chickens (Sedghi et al. 2010; Moradi et al. 2014).

16 Effect on triglyceride, cholesterol, very low-density lipoprotein, low-density lipoprotein, and high-density lipoprotein concentrations

Moradi et al. (2017) reported that giving broiler chickens LE in their water dramatically decreased their serum low-density lipoprotein (LDL) cholesterol and total cholesterol, while Al-Sofee (2018) reported the same for Japanese quail. Feeding Japanese quail, a powder made from liquorice roots, decreased their triglyceride and cholesterol levels (Myandoab and Hosseini 2012). Rezaei et al. (2014) found that supplementing broiler chicken feed with *G. glabra*, *Thymus vulgaris*, or both herbs, remarkably reduced the birds' serum triglyceride levels. Cholesterol and LDL cholesterol levels fell when Liquorice and a prebiotic mixture were added to the feed of broiler chickens (Sedghi et al. 2010), but there was little to no effect on blood triglycerides, VLDL, or HDL cholesterol levels. Liquorice has been linked to a reduction in LDL cholesterol because it prevents the oxidation of LDL cholesterol by blocking cyclooxygenase (COX) and lipoxygenase (LPX) enzymes (Abdul Majeed 2019). In another study, adding liquorice root powder to the diets of broiler chickens resulted in a considerable rise in plasma HDL cholesterol while not affecting plasma LDL cholesterol, total cholesterol, or triglycerides (Gowthaman et al. 2021). The quail fed a 750-mg licorice diet had lower contents of all lipid profile parameters (LDH, TC, TG, and LDL) (Reda et al. 2021).

17 Effect of Liquorice on other blood parameters

Supplementation with Liquorice Broiler hens showed elevated levels of uric acid, alkaline phosphatase (ALP), aspartate aminotransferase (AST), calcium, and phosphorus, as well as RBCs, WBCs, platelets, erythrocyte sedimentation rate (ESR) and plasma uric acid (Al-Daraji 2012). Amen and Muhammad (2016) discovered a similar effect when they fed LE to broiler chickens; they saw a rise in platelets, haemoglobin, WBCs and RBCs. However, Al-Sofee (2018) found that supplementing Japanese quail with licorice root had no appreciable effect on their packed cell volume (PCV). Also, giving broiler chickens Liquorice increased their white blood cell count (Sedghi et al. 2010), and giving Japanese quail LE in their water increased their triiodothyronine (T3) and thyroxine/ tetraiodothyronine (T4) levels (Hosny et al. 2020). However, a different study on broiler chickens at six weeks of age reported no significant changes in serum WBC count or cell-mediated immunity (Jagadeeswaran et al. 2012). Liquorice extract can reduce malondialdehyde's concentration in broiler chicken breast meat experimentally exposed to aflatoxin-B₁ (Rashidi et al. 2020).

Conclusion and future prospects

Liquorice contains bioactive compounds with powerful pharmacological and therapeutic effects, such as flavonoids and glycyrrhizin. It has been hypothesized that LE's immunogenic and antioxidant properties could make it an effective alternative to antibiotics for treating and preventing respiratory, gastrointestinal, and immunological diseases in poultry. To increase productivity and bird health, however, better distribution of this essential herb is required in poultry. This review has also postulated that *G. glabra* supplementation to poultry diets may enhance development, productivity, feed efficiency, carcass characteristics, and meat quality. Furthermore, the liquorice herb's excellent role in increasing the activities of the digestive system may treat several illnesses and issues in chicken farms. Standardizing the use of Liquorice (in water or feed) is important to guarantee consistent outcomes and appropriate dosing. Therefore, this standardization should be the focus of future studies if Liquorice is to be used effectively in the poultry sector.

Glycyrrhiza glabra extract may be a key component in creating numerous pharmaceutical compounds useful in the poultry industry. Liquorice has medicinal properties because of the diverse bioactive components it contains. The immunogenic and antioxidant effects of licorice extract improve the growth performance, carcass characteristics, feed conversion efficiency and hemato-biochemical indicators of poultry and have potential biomedical applications for a wide range of digestive, respiratory and immunological ailments. Poultry that consumed water containing up to 0.4 g/L of LE had better immunological

responses, higher antioxidant indices, and a healthier lipid profile. When added to the diets of laying hens, 50 g/mL of LE has been shown to affect cellular immunity positively. Extraction of *G. glabra* at 300 g/mL was highly effective against NDV. Additional study is required to evaluate the benefits of licorice herb as a chicken feed supplement and explore different characteristics of this medicinal plant that can enhance poultry productivity and health. *In ovo* delivery and nano delivery, strategies should be researched to optimize the delivery of this important herb in poultry, which could increase productivity and safeguard the health of the poultry birds.

Acknowledgement

All the authors acknowledge and thank their respective Institutes and Universities.

Author's contribution

All the authors contributed significantly.

Funding

This compilation was written by its authors and required no substantial funding.

Disclosure statement

All authors declare that there exist no commercial or financial relationships that could, in any way, lead to a potential conflict of interest.

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