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Liquorice *(Glycyrrhiza glabra)* herb as a poultry feed additive- A review

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Abstract

Medicinal plants have grown in popularity as a result of their numerous health benefits in animals, birds, and humans. Liquorice (*Glycyrrhiza glabra*) is a prominent traditional medicinal plant belonging to the *Fabaceae* family of legumes. The principal constituent of liquorice root is glycyrrhizin. Liquorice extract have been noted to have a significant antimicrobial (antifungal, antibacterial, antibiotic, antiviral, antiseptic, and antiprotozoal) effects. Improved feed conversion ratio, average body weight, and feed intake have been observed in quails supplemented with dietary 200 ppm of liquorice root extract and 1% probiotic in their diet. The liquorice extract has been found to have immunogenic and antioxidant properties, which could help poultry improve their growth performance, feed efficiency, carcass characteristics, and serum biochemistry, and can potentially act as a viable alternative to antibiotics in the treatment and prevention of various respiratory, digestive, and immune disorders.

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1. Introduction

Medicinal plants have grown in popularity as a result of their numerous health benefits in animals, birds, and humans. The use of feed additives and nutritional supplements in the chicken diets, such as prebiotics, plant extracts, and probiotics, is gaining popularity these days due to their numerous benefits in terms of improving development and production while also protecting poultry health (Alagawany et al. 2019). The use of herb liquorice (*Glycyrrhiza glabra*) as a feed additive in poultry is the subject of this review. *Glycyrrhiza glabra* is a prominent traditional medicinal plant from *Fabaceae* family of legumes. *Glycyrrhiza glabra*, usually known as liquorice, is a traditional crop with whom health benefits and medicinal have been associated since centuries ago (Karkanis et al. 2018). Glycyrrhizin is the primary and main active component in liquorice root which is a triterpene saponin that is several times sweeter than sucrose (Pastorino et al. 2018). Liquorice is used in so many conditions like arthritis, mouth ulcers and also have potential anti-inflammatory, antineoplastic, immuno-modulatory, antioxidant, antimicrobial, detoxifying, and growth promoting effects (Alagawany et al. 2019). The composition, health benefits, and functional applications of the liquorice herb are described in this review for a wide range of audience. As a result, one can safely examine and gain a new perspective on the benefits of liquorice in poultry nutrition, its impacts on poultry development, and productivity, as well as their immunological and antioxidant status.

2. Plant description and morphology

Liquorice is one among the well-known herbs around the world, basically used for therapeutic purposes (Shebl et al. 2012). In the family *Leguminosae*, the Glycyrrhiza genus has nearly 30 species including *G. uralensis*, *G. glabra*, and *G. inflata* (Nomura et al. 2002). Liquorice plant is herbaceous perennial, growing to up to 2.5 m in height. The bark of the liquorice root is dark-red and inside it is bright-yellow. It is characterised by alternating leaves, pinnate, with ovate nine to seventeen yellow-green leaflets, each 2.5 to 5 cm long. Pea-like blossom spikes can be purple, white or yellow. Spikes are commonly 10 to 15 cm long and born from axil leaves. Seedpods are maroon, elongated, three cm long, pointed, and flat. Liquorice roots are harvested normally three to four year after planting (Mamedov and Egamberdieva 2019).

3. Origin/Distribution

Glycyrrhiza glabra/Liquorice is a perennial herb, also known as mulethi, which is native to West Asia, North Africa, and Eurasia (Pastorino et al. 2018). Liquorice shows wide distribution in Asia (n.w India, China, Armenia, Pakistan Azerbaijan, Kazakhstan Georgia, Russia, Tajikistan, Iran, Mongolia, Turkmenistan, Kyrgyzstan, Uzbekistan, Palestine, Jordan, Iraq, Syria, Turkey, Lebanon, Afghanistan), Africa (Libya), and Europe (Bulgaria, Moldova, Albania, Italy, Romania, Ukraine, Former Yugoslavia, Greece, France) (Al-Snafi 2018).

4. Common names

The genus name, Glycyrrhiza, was derived from Greek words glykys (means sweet), and rhiza (means root). The species name, glabra, was derived from Latin word glaber, which means bald or smooth and refers to smooth husks. The common names of liquorice are:- Arabic: rib el-sus, Irik Sus, Sus; English: licorice-root, licorice, liquorice; German: Lakritze, Subholz; French: reglisse; Swedish: lakritsrot; Italian: liquirizia; Spanish: orozuz, regaliz, alcazuz, licorice (Al-Snafi 2018).

5. Chemical composition and bioactive compound of Liquorice

Liquorice is a main source of polysaccharides and simple sugars, proteins, mineral salts, pectins, sterols, resins, and gums (Wang et al. 2015). Biological compounds such as saponins, flavonoids, and triterpenes have been isolated from liquorice (Wang et al. 2015, Farag et al. 2012). Saponins are the reason behind the sweet taste of liquorice. The content of these compounds can vary considerably as a result of different processing methods, geographical sources, and harvesting methods. The principal constituent of liquorice root is glycyrrhizin. The highest level of glycyrrhizinic acid (GRA) is seen in the main root followed by lesser content in lateral roots, while the plant parts above ground do not contain GRA and are usually regarded as waste (Fuggersberger-Hein and Franz 1984). Glycyrrhizin, rich in magnesium, potassium and, calcium salts, constitutes about 10% of dry weight of liquorice root (Rizzato et al. 2017). The content of glycyrrhizin in liquorice is species specific and ranges from 2 to 25%. Liquorice extract (LE) has been used as a sweetener (Hayashi and sudo 2009) because glycyrrhizin is nearly 50 times sweeter than sucrose (cane sugar) (Omar et al. 2012). The proximate composition of liquorice is moisture 20%, sugars 3-16 %, starch 30%, glycyrrhizin 5-24 %, and ash 6% but based on the growth, environment, and cultivation conditions the composition varies (Fenwick et al. 1990; Vlaicu et al. 2021).

In addition, 49 phenolic compounds and 15 different saponins (including their glycosides) have been identified in liquorice root (Kitagawa 2002). Presence of yellow colour in liquorice is because of flavonoid content and the flavonoids belong to various classes, including isoflavanes, flavones, flavanones, chalcones, flavanonols, isoflavenes, isoflavanones, and isoflavones. The main flavonoids are iso liquiritigenin (2', 4, 4'-tri-hydroxychalcone) and glycoside liquiritigenin (4',7-dihydroxyflavanone) such as isoliquiritin, liquiritin apioside, liquiritin, and licuraside (Pastorino et al. 2018). Glabridin is the main isoflavone reported, ranging between 0.08 to 0.35% of the root dry weight (Simmler et al. 2013). Interestingly, high-performance liquid chromatography analysis of methanolic extract revealed the presence of organic acids like fumaric, citric, acetic, propanoic, malic, butyric, and tartaric acid in liquorice (El-Saber et al. 2020).

6. Traditional uses

Liquorice has a long history of therapeutic use in Asia and Europe. Fresh leaves of liquorice were used for treatment of external wounds. Root and rhizome were orally used to treat diabetes, kidney stones, cystitis, gastric ulcers, tuberculosis, cough, Addison's disease, and stomach-ache. It has been used as contraceptive, mild laxative, and has also been used to improve sexual function of male (Asl and Hosseinzadeh 2008). Liquorice root has shown application in the treatment of arthritis, lung diseases, heart diseases, eczema, kidney diseases, low blood pressure, gastric ulcer, liver toxicity, allergies, and some microbial infections (Mamedov and Egamberdieva 2019). It is mainly used in medicinal sector and as a food preservative or flavouring agent for commercial purposes (Pastorino et al. 2018).

7. Pharmacological activities of Liquorice

7.1 Antibacterial activity

The methanolic extracts of liquorice roots have shown substantial antibacterial activity *in vitro* against a variety of bacteria, including *Bacillus cereus*, *Agrobacterium tumefaciens*, *Pseudomonas syringae* pv. tomato and *Bacillus subtilus*. Water extracts, on the other hand, showed no such action (Karkanis et al. 2018). In an experiment, ethanolic extracts of liquorice leaves were found to be effective against *Candida albicans*, *Bacillus subtilis*, and *Staphylococcus aureus* (Irani et al. 2010). While liquorice root extracts in chloroform, ether, and acetone were found to be effective against two gram-negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*) and two gram positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) (Nitalikar et al. 2010). Glycyrrhizic acid (GRA) obtained from liquorice have antibacterial activity against *P. aeruginosa* (Chakotiya et al. 2016).

7.2 Antiviral activity

In *in vitro* experiments the antiherpetic effect of aqueous extracts of liquorice roots against Herpes Simplex Virus 1 has been reported and this antiherpetic effect could be attributed to a variety of mechanisms, including the role of *G. glabra* in inhibiting HSV attachment via direct contact with the extract. HSV-1 inhibition is also mediated by either virus inactivation or the antiadhesive feature of *G. glabra* aqueous extract, which prevents HSV-1 from adhering to Vero cells *in vitro* (Sabouri Ghannad et al. 2014). Antiviral activity of *G. glabra* extracts have also been proven against New castle disease virus (NDV) (Omer et al. 2014). Dietary inclusion of LE has been shown to inhibit the replication of Paramyxovirus type 1 (PPMV-1) in pigeons (Dziewulska et al. 2018). Treatment with glycyrrhizin alone or Duck Hepatitis virus (DHV) vaccine with glycyrrhizin has shown significant antiviral effects against the DHV and glycyrrhizin turned out to be a good immune stimulant (Soufy et al. 2012).

7.3 Antioxidant activity

Substantial antioxidant effects of liquorice have been demonstrated under *in vivo* (Fuhrman et al. 1997; Kuhnl et al. 2015) and *in vitro* (Vaya Belinky and Aviram 1997; Tohma and Gulcin 2010; Fu et al. 2013; Martins et al. 2015) conditions. Vaya Belinky and Aviram (1997) identified seven compounds with antioxidant action against the oxidation of low-density lipoproteins (LDL), with glabridin to be the most powerful antioxidant. Another study examined the antioxidant ability of liquorice extracts from several species for both lipid peroxidation inhibition (LPI) and ABTS-+ radical scavenging, and it was found that the antioxidant ability was dose-dependent, particularly for lipid peroxidation (Fu et al. 2013).

7.4 Anti-inflammatory activity

Liquorice extracts or particular ingredients have shown significant anti-inflammatory properties under *in vitro* (Fu et al. 2013) as well as *in vivo* (Shah et al. 2018) conditions. The flavonoids isolated from liquorice extracts exert anti-inflammatory effects by reducing the production of nitric oxide, interleukin-6, and prostaglandin E2 in LPS-induced macrophage cells (Fu et al. 2013). Similar results were reported with Licochalcone A (Lico A), a flavonoid derived from liquorice, which exerted significant anti-inflammatory properties in both *in vitro* and *in vivo* models produced by lipopolysaccharide (LPS) (Chu et al. 2012).

7.5 Other pharmacological effects

Other pharmacological effects of liquorice are antiulcer (Krausse et al. 2004), antitussive (Kuang et al. 2018), antitumor (Dong et al. 2007), and hepatoprotective (Li et al. 2019) activities, which have been studied under *in vitro* and *in vivo* conditions where liquorice extracts as well as isolated bioactive compounds were used. Krausse et al. (2004) reported that GRA has anti *Helicobacter pylori* activity and beneficial effects on peptic ulcers. Liquiritin and liquiritin apioside are the important expectorant and antitussive compounds of liquorice (Kuang et al. 2018). Glycyrrhizin has shown activity against the Hepatitis C virus by which it has shown hepatoprotective benefits and also protect liver from oxidative damage (Ashfaq et al. 2011; Rasool et al. 2014).

8. Beneficial role of liquorice in poultry

Liquorice supplementation as a feed additive showed antiviral activity against NDV (Omer et al. 2014). Ocampo et al. (2016) reported that the broiler chicken fed GRA at the level of 60 μg per mL drinking water resulted in increased antibody titers against NDV. Dziewulska et al. (2018) reported that dietary inclusion of LE inhibit the paramyxovirus type 1 (PPMV-1) replication in pigeons by reducing viral RNA copy numbers which suggests that liquorice has antiviral effects. Glycyrrhizin has been reported to inhibit the uptake of influenza virus into cell (Wolkerstorfer et al. 2009). The LE has been noted to have a significant antimicrobial (antifungal, antibacterial, antibiotic, antiviral, antiseptic, and antiprotozoal) effect (Nitalikar et al. 2010; Chakotiya et al. 2016; Fatima et al. 2009; Sabouri Ghannad et al. 2014; Martins et al. 2016) antineoplastic, anti-inflammatory, and anti-oxidant effects (Martins et al. 2015). It acts as a growth promotor in chicken and is also used in conditions like oral ulcers and joint pains (Alagawany et al. 2019; Karkanis et al. 2018).

Abdominal fat in broiler chicken decreased significantly with the supplementation of liquorice (2 g/kg) in broiler chicken feed (Sedghi et al. 2010). Significant improvement in body weight gain of broiler chicken at six weeks of age was observed by dietary supplementation of 1 g LE/kg diet (Amen and Muhammad 2016). However, at higher inclusion levels decrease in body weight gain was observed compared to control group. Supplementation of poultry diet with *G. gabra* was shown to exert a significant ($P<0.05$) positive effect on the growth performance of broiler chicken (Alagawany et al. 2019). The supplementation of GRA in broiler chicken diets (60 μg/mL) significantly improved the final body weight, average body weight gain, feed conversion ratio (FCR), and livability compared to control birds (Ocampo et al. 2016). In another study, improved body weight gain of broiler chicken reared at high stocking density was observed when feed was supplemented with 500 ppm LE but the feed efficiency of birds remained unaffected (Rashidi et al. 2019).

9. Effect of Liquorice on growth performance

Experiments on the dietary addition of liquorice root were conducted mostly in broiler chicken and Japanese quail. Best performance results have been observed in broiler chicken by supplementation of liquorice at the level of 2.5 g/kg diet (Hosseini et al. 2011). The supplementation of LE in quails via drinking water at the levels of 100 mg and 450 mg/L improved the final body weight and body weight gain of birds in a dose dependent manner (Hosny et al. 2020). However, these effects of LE supplementation were observed in male birds only. Similarly, a decrease in FCR and increase in average body weight and feed intake of quails supplemented with 200 ppm liquorice extract and 1% probiotic was reported (Myandoab and Hosseini 2012). An improved growth performance of broiler chicken was also reported on dietary supplementation of garlic and liquorice mixture (Al-Zuhairy and Hashim 2015). The supplementation of aqueous LE in drinking water of broiler chicken resulted in significant increase of body weight gain, whereas, water intake, feed intake, and feed conversion efficiency of birds remained unaffected (Beski et al. 2019).

However, contrary to the above observations the supplementation of glycyrrhiza in broiler chicken reduced the feed consumption and body weight gain of birds compared to control (Rezaei et al. 2014). The decrease in body weight has been attributed to liquorice flavonoids which have the potential to reduce the body fat content (Tominaga et al. 2006). Furthermore, the decline in growth performance at higher inclusion levels of herbal plants has also been ascribed to higher crude fiber levels, which potentially creates nutritional imbalances and negatively affects body metabolism (Vlaicu et al. 2021). Most of the studies have reported that liquorice root extract supplementation in broiler chicken have no significant effects on the body weight, feed intake, and feed efficiency of birds (Sedghi et al. 2010; Salary et al. 2014; Moradi et al. 2017). In Japanese quail, the supplementation of liquorice root powder had no significant effects on the final body weight, weight gain, feed intake, feed efficiency, livability, and egg weight (Al-Sofee et al. 2018; Dogan et al. 2018b).

10. Effect on carcass quality and yield

The availability of literatures related to effects of feeding the liquorice plant on carcasses and meat quality is considerably limited. Moradi et al. (2017) stated that abdominal fat decreased significantly in broiler chicken supplemented with 0.3 g liquorice/L water and this decline was attributed to the constituent hydrophobic flavonoids found in liquorice (Nakagawa et al. 2004). Though, abdominal fat in broiler chicken decreased significantly when drinking water was supplemented with 0.45 g liquorice extract/L water, no change was observed in the carcass yield of chicken raised under heat stress (Al-Daraji 2012). However, LE supplementation in broiler chicken had no significant effects on the relative weights of internal organs such as bursa of fabricius, thymus, spleen, liver, and heart (Sedghi et al. 2010; Moradi et al. 2014). Similarly, no significant effect was observed in the weights of spleen and gizzard of Japanese quail due to 200 ppm LE supplementation, though higher relatives weights of liver were observed (Myandoab and Hosseini 2012). The supplementation of aqueous LE in drinking water of broiler chicken did not reveal any significant effect on the intestinal histomorphology, visceral organs, and carcass cuts (Beski et al. 2019). In Japanese quail, the supplementation of liquorice root extract resulted in significant increase in percentage carcass yield (Myandoab and Hosseini 2012), whereas, in another study LE supplementation in Japanese quail had no significant effect on the carcass percentage (Hosny et al. 2020). The supplementation of glycyrrhiza glabra decreased weights of pancreas, liver, abdominal fat, bursa, and increase in spleen weight was noticed (Rezaei et al. 2014). However, Kalantar et al. (2017) reported that supplementation of liquorice resulted in significant increase in the immune organs weight such as bursa and spleen of broiler chicken. One of the main pharmacological actions of Liquorice is its antioxidant action (Michaelis et al. 2011) which has been associated with improvements in carcass traits and quality of poultry meat as a result of its influence on the fat and protein metabolism.

11. Effect of Liquorice on some blood parameters

11.1 Effect on blood glucose

The dietary supplementation of liquorice root extract or powder in broiler chicken and Japanese quails increased plasma glucose level in a dose dependent manner (Al-Daraji 2012; Dogan et al. 2018a; Dogan et al. 2018b). This increase in glucose level may be attributed to glycyrrhizin, a saponin glycoside, that is found to be sixty times sweeter than sucrose (Roshan et al. 2012). However, in contrast few studies have reported decreased serum glucose level in broiler chicken supplemented with LE (Moradi et al. 2017) or *Glycyrrhiza glabra* (Rezaei et al. 2014). The supplementation of LE has not shown any significant effect on the plasma glucose level of broiler chicken (Sedghi et al. 2010) and Japanese quail (Myandoab and Hosseini 2012; Al-Sofee 2018; Abdul-Majeed 2019).

11.2 Triglyceride, cholesterol, VLDL, LDL, and HDL concentrations

In broiler chicken the supplementation of LE in drinking water resulted in significant reduction in serum LDL cholesterol and total cholesterol (Moradi et al. 2017) and similar results were observed in Japanese quail (Al-Sofee 2018). In Japanese quail, the dietary supplementation of liquorice root powder at different levels resulted in a significant decrease of triglyceride and cholesterol level compared to the control birds (Abdul-Majeed 2019; Myandoab and Hosseini 2012). The dietary supplementation of *Glycyrrhiza glabra*, *Thymus vulgaris* or their mixture in the broiler chicken significantly reduced the level of serum triglycerides (Rezaei et al. 2014). Furthermore, supplementation of liquorice and prebiotic mixture in feed of broiler chicken exerted no significant effects on serum triglyceride, VLDL, and HDL concentration, whereas, cholesterol and LDL cholesterol concentration decreased in response to liquorice supplementation (Sedghi et al. 2010). This decrease of LDL cholesterol has been attributed to the action of liquorice that protects LDL cholesterol from oxidation by inhibiting the enzymes like cyclooxygenase and lipoxygenase which in turn inhibit lipid peroxidation (Craig 1999). In another study, the supplementation of liquorice root powder at different levels in chicken diet resulted in significant increase in plasma HDL cholesterol, while no significant changes were observed in plasma LDL cholesterol, total cholesterol, and triglyceride in broiler chicken (Dogan et al.

2018a).

11.3 Heterophils, lymphocytes and H/L ratio

The H/L ratio is considered as a reliable indicator of heat stress in chicken (Gross and Seigel 1983) and heat stress has been demonstrated as a major stressor which brings about changes in heterophils and H/L ratio in chicken (Yalcin et al. 2013; Habibian et al. 2014) and Japanese quail (Mahmoud et al. 2013). The supplementation of LE in drinking water has significantly decreased the H/L ratio of broiler chicken raised under heat stress (Al-Daraji 2012). However, in other studies the supplementation of LE in broiler chicken did not influence the percentage of lymphocytes, heterophils, monocytes, or H/L ratio (Moradi et al. 2014; Sedghi et al. 2010).

11.4 Total Proteins (TP)

In Japanese quail the dietary supplementation of crushed liquorice root significantly increased the serum TP level (Al-Sofee 2018). Similar results were obtained in broiler chicken by supplementation of *G. glabra* in chicken diets (Rezaei et al. 2014). However, in contrast to these observations in another study no change in serum total protein levels were observed in Japanese quail supplemented with liquorice root powder (Abdul-Majeed 2019).

11.5 Other blood parameters

In broiler chicken significant increase in the WBC, RBC, PCV, plasma uric acid, alkaline phosphatase, aspartate aminotransferase, calcium, and phosphorus were observed under liquorice supplementation (Al-Daraji 2012). On similar lines, the LE supplementation in broiler chicken resulted in increase of WBC, RBC, PCV, and Hb concentration (Amen and Muhammad 2016). However, no significant effect of dietary supplementation of liquorice root was observed on the PCV of Japanese quail (Al-Sofee 2018). Furthermore, liquorice supplementation increased the WBC count of broiler chicken (Sedghi et al. 2010) and LE supplementation in water increased the T3 (Tri iodothyronine) and T4 (Thyroxin) levels in Japanese quail (Hosny et al. 2020). However, in another study on broiler chicken no significant effects were observed on the serum albumin, globulin, albumin:globulin ratio, WBC count, and cell mediated immunity at six weeks of age (Jagadeeswaran et al. 2012).

12. Conclusion

Bioactive components found in liquorice include flavonoids and glycyrrhizin, which exert profound pharmacological and therapeutic activities. The liquorice extract has been found to have immunogenic and antioxidant properties, which could help poultry birds improve their growth performance, feed efficiency, carcass characteristics, and serum biochemistry, and can potentially act as a viable alternative to antibiotics in the treatment and prevention of respiratory, digestive, and immune disorders. However, dedicated and sincere efforts are required to improve the delivery of this vital herb in poultry to achieve more efficient production and enhanced bird health.

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