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WITHANIA SOMNIFERA AS A PHYTOHERBAL GROWTH PROMOTER FOR BROILER FARMING – A REVIEW*

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Abstract

Poultry production significantly contributes to bridging the food gap worldwide. Several nations have limited the usage of antibiotic growth promoters due to bacterial resistance to antibiotics and the occurrence of residual antibiotics in the edible tissues of birds. The world is now turning to using natural alternatives to increase poultry production as well as birds' resistance to diseases. *Withania somnifera* (WS; family: Solanaceae) is a precious medicinal herb utilized in several countries due to its distinct chemical, medicinal, and physiological properties. This plant has antioxidant, anti-aging, antimicrobial, antitumor, hepatoprotective, cardioprotective, neuroprotective, immunomodulatory, antidiabetic, antistress, and growth-promoting activities. In poultry, the dietary inclusion of WS revealed a promising result in enhancing productive performance, increasing disease resistance, reducing stress effects, and maintaining the bird's health. Thus, the current review highlights the morphological features, distribution, chemical structure, and pharmacological features of *Withania somnifera* as a growth promoter herb for farming broiler chickens.

Key words: adaptogen plant, herbal extracts, poultry productivity

Nowadays, world food production depends mainly on animal protein. In many nations, the poultry business has grown as a source of high-quality meat and eggs to help balance human food (Kralik et al., 2018; Réhault-Godbert et al., 2019). The nutritional and economic demands of nations for a poultry-based diet have forced the intensive production of poultry (Haque et al., 2020). Poultry production is considered the most competitive and rapidly growing section of the animal husbandry business. The increase in poultry meat production is a result of intensive poultry farming technologies (Mottet et al., 2017).

The poultry industry faces substantial challenges, in terms of having safer products without antimicrobial residues and environmentally sustainable poultry production. This has triggered the discovery and spread of the application of various feed additives that help enhance productivity, reduce pathogens colonization, and lower mortality in the poultry industry (Salim et al., 2018; Hafez and Attia, 2020; Krysiak et al., 2021).

The recent substitution of antibiotic growth promoters (AGPs) with natural substances in avian ration acquired much attention (Selaledi et al., 2020). However, numerous natural growth promoters, including phytogenic products, herbal extracts, yeasts, probiotics, prebiotics, symbiotics, essential oils, and organic acids, have been known to be effective and safe alternatives to AGPs (Sethiya, 2016; Lillehoj et al., 2018; Salim et al., 2018; Bajagai et al., 2020; Bilal et al., 2021).

The application of herbs as growth promoters in animal feed is becoming common worldwide due to their antimicrobial, anti-inflammatory, antioxidant, and immunostimulant properties as well as their improving feed efficiency, nutrient digestibility, and animal performance (Suganya et al., 2016). Moreover, traditional medicinal herbs can combat the negative effects of heat stress on the productivity of broiler chickens (Owen, 2011).

Among these medicinal plants, *Withania somnifera* L. Dunal (WS), which is commonly known as Ashwa-

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gandha in Sanskrit or Indian Winter Cherry or Indian Ginseng in English, is an evergreen woody shrub of the Solanaceae family. The species name *somnifera* means sleep-inducer, an anti-stress characteristic (Paul et al., 2021). It is found in the Canary Islands, a Mediterranean region through Africa, the Middle East, India, and Sri Lanka to China (Gurib-Fakim, 2008).

W. somnifera is a phytoherb with several effects, including anabolic, hypolipidemic, antistress, and antioxidant effects (Verma et al., 2012), immunomodulatory, antitumoral (Ahmed and El-Darier, 2024), and anti-inflammatory activities (Hussain et al., 2021), ability to stimulate bone mineralization (Nagareddy and Lakshmana, 2006), chondroprotective effects (Sumantran et al., 2007; Shubhashree et al., 2018), and ability to increase hemoglobin level, platelet, erythrocyte, and white blood cell counts (Tharakan et al., 2021). Moreover, WS can improve circulating cortisol, accelerate physical performance, reduce fatigue, and lower refractory depression in animals subjected to variable stressors (Singh et al., 2001, 2003). Similarly, WS is also thought to stimulate the immune system in stressed birds (Marimuthu et al., 2020). This review sheds light on the morphological features, distribution, chemical structure, and pharmacological features of *Withania somnifera* as a growth promoter herb for broiler chicken farming.

Morphological characteristics, distribution, chemical composition, and common use of *W. somnifera*

W. somnifera (L.) Dunal (WS) is the most abundant in the genus. It is an erect, evergreen, branching, tomentose shrub growing about 30–150 cm in height. Leaves are simple, petiolate, and alternate flowering shoots (Mirjalili et al., 2009; Aslam et al., 2017; Paul et al., 2021), flowers are greenish-yellow, pedicels are up to 4 mm in length and 4–6 mm in diameter and axillary, and umbellate cymes occur in 5–25 clusters. Fruits are red-yellow, globose, 5 mm in diameter, and enclosed in the globose calix containing the seeds (Rajeswara et al., 2012).

Due to the economic and medicinal properties of *W. somnifera* (Sengupta et al., 2018) and the attractive price for roots, this genus, with 26 species (Rajeswara et al., 2012), is widely spread mainly in China, Asia, Africa, Australia, and Europe (Aslam et al., 2017; Afewerky et al., 2021).

The chemical composition of WS has been broadly investigated, and over 35 chemical agents have been found and extracted, including 12 alkaloids, 35–40 withanolides, and several sitoindosides (Barthi et al., 2016). The withanolides are naturally found steroidal lactones that impart a distinctive earthy odor and flavor to Ashwagandha (Sangwan et al., 2017). Withaferin A was the primary isolated member of this group in 1965 (Bonandi et al., 2021).

W. somnifera roots present 0.13–0.31% of alkaloids. However, higher values (up to 4.3%) have been reported (Mirjalili et al., 2009). The root's composition also consists of a trim level of soluble protein (5.6%) (Verma and Gaur, 2011).

The alkaloid group comprises withanine, withananine, pseudo-withanine, somniferine, somnine, somniferinine, choline, cuscohygrine, isopelletierine, anaferine andanahydrine, tropine, pseudo-tropine, and 3-a-gloyloxytropane (Singh et al., 2010; Narinderpal et al., 2013; Ali et al., 2020). The steroidal lactones of WS are illustrated in Figure 1.

According to Kirson et al. (1971), withanolides are produced via the oxidation of steroids. These compounds are localized in different amounts in the plant parts (Sangwan et al., 2004), mainly in leaves varying from 0.001 to 0.5% of dry weight (Dhar et al., 2015), in comparison with 0.023% of dry weight in the root (Gaurav et al., 2016).

In humans, the WS is traditionally utilized due to its aphrodisiac, sedative, rejuvenating, energy-enhancing, and life-prolonging effect (Mirjalili et al., 2009). However, the plant still exhibits high anti-inflammatory activity associated with steroid content, withaferin A, the main component of those steroids (Jana and Charan, 2018). The anti-inflammatory activity is similar to glucocorticoid hydrocortisone (Narinderpal et al., 2013).

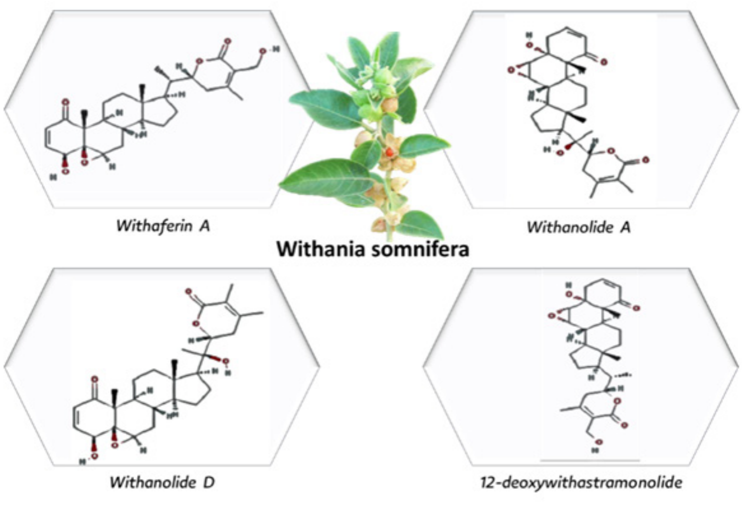

Figure 1. The steroidal lactones of *Withania somnifera*

Antimicrobial effects against fungi, viruses, and bacteria, including *P. aeruginosa, E. coli, S. aureus*, and *Candida albicans,* hepatitis C virus, were reported by Singh and Kumar (2011), El-Boshy et al. (2013), and Mofed et al. (2020). *W. somnifera* is also a promising alternative in protecting against SARS-CoV-2 (Kumar et al., 2020; Balkrishna et al., 2021; Chickhale et al., 2021).

There is evidence that thyroid activity is normalized in rats with induced hypothyroidism as a result of using the methanolic extract of WS (Sharma et al., 2018; Abdel-Wahhab et al., 2019).

Studies were performed to estimate the toxic effects of WS. Prabu et al. (2013) and Patel et al. (2016) administered 2000 mg/kg/day of hydroalcoholic extract of WS root to rats. No signs of significant toxicity were observed since the animals had no mortality or hematological, biochemical, or histopathological changes.

Pharmacological activities of *W. somnifera*

The pharmacological activities of WS are briefly illustrated in Figure 2.

Antioxidant activity

The antioxidant activity of WS has been detected mainly in leaves compared to bark, stem, and roots and is due to the high content of phenolics, flavonoids, and other pigments in the leaves (Fernando et al., 2013; Azimi et al., 2020; Hassanin et al., 2020; Saggam et al., 2021) as it elevates the levels of naturally found antioxidant enzymes (Dhuley, 2000).

In a study with rats that were aluminum-intoxicated and treated or not with 200 mg/kg extract of WS daily, Elhadidy et al. (2018) recorded non-significant changes in the activity of enzymes, acetylcholinesterase and glutathione, and lipid peroxidation in cortex and hippocampus in comparison with the non-intoxicated rats.

Stressed equines were treated with root extract of WS for 21 days, and it was observed that treated animals had a reduction in the concentrations of glucose, triglycerides, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and TBARs. Moreover, an augment was observed in the glutathione and superoxide dismutase levels, indicating a hepatoprotective and renoprotective effect (Priyanka et al., 2020).

Improvements in IgA, IgG and IgM, T cells, B cells, and NK cells, higher counts of platelet, total leukocytes, neutrophils, and lymphocytes, and lower LDL concentrations were associated with the humans' usage of WS (Tharakan et al., 2021).

Several studies indicated a beneficial effect of the WS extract on the oxidative status of broilers, with an improvement in activities of glutathione peroxidase and superoxide dismutase and lower liver peroxidation due to its content of biologically active phytochemicals (Ahmed et al., 2015; Vasanthakumar et al., 2015; Azimi et al., 2020).

Immunomodulatory activity

W. somnifera is a potent immunostimulant (Saggam et al., 2021), which raises the humoral and cell-mediated immune response (Verma et al., 2012).

According to Siddiqui et al. (2012), WS has two major withanolides, withaferin A and withanolide D, which may modulate the immune system influencing T cell proliferation, enhancing neutrophil numbers, and producing considerable humoral response against sheep red blood cells.

Immune responsiveness against pathogens may be enhanced by immunomodulatory agents of vegetal and animal origin through the improvement of the non-specific immune response (Arora et al., 2021), and WS may be utilized as an immunomodulatory agent as it increases phagocytic activity and IgG levels in mice (Kanyaiya et al., 2014).

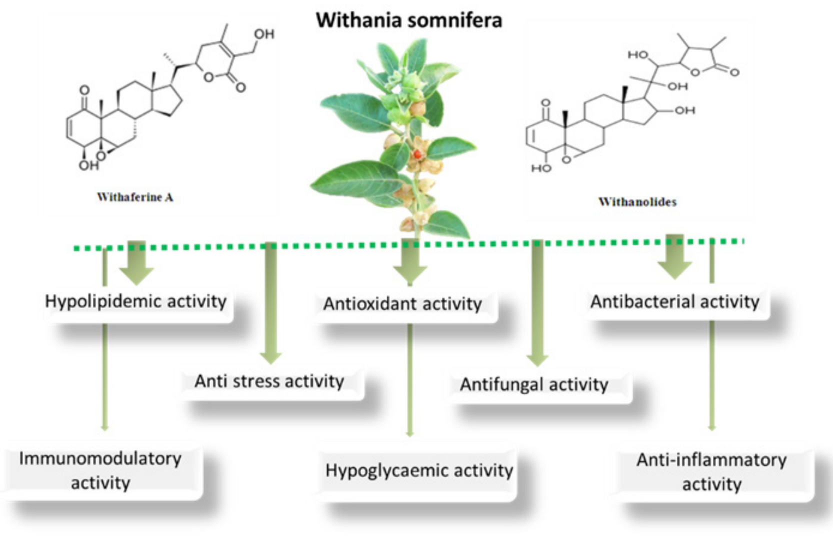

Figure 2. The pharmacological activities of *Withania somnifera*

Reduction in interleukin-6 level and increase in red blood cell count, total leukocyte count, and hemoglobin levels were recorded in equines treated with root extract of *W. somnifera* (Priyanka et al., 2020). In an *in vitro* study, Arora et al. (2021) assessed the immunomodulatory impacts of the tannins from the WS root. They demonstrated that tannins stimulated the phagocytic activity of neutrophils and increased neutrophil motility because tannins act as a chemoattractant.

T and B cells are major effectors of the adaptative immune system and are present throughout the spleen (Lewis et al., 2019). Thus, its higher weight is a sign of increasing the immune cell production and cell population (White et al., 1975).

The higher lymphoid organs weight and humoral immune response in broilers supplied with feed, containing 0.5 and 1% WS powder, were reported by Tomar et al. (2018). However, Kumar et al. (2018 b) did not observe the effects of WS extract on spleen weight and NDV titers with 50 or 100 mg WS extract in the broiler's diet. As reported by Mirakzehi et al. (2017), using WS root extract (150 mg/kg diet) may enhance humoral immunity, which increases IgG levels in 42-day-old broilers, probably as a result of the higher spleen weight.

Anti-inflammatory activity

W. somnifera is a natural anti-inflammatory steroid that has a potent anti-inflammatory effect (Saggam et al., 2021) due to its flavonoid content. Flavonoids have antioxidant and radical scavenging activity (Heim et al., 2002), regulate activities of inflammation-related cells, inhibit T cell proliferation, modulate the activity of the enzymes lipoxygenase, cyclooxygenase, and phospholipase A_2, and lower the formation of arachidonic acid, leukotrienes, and prostaglandins, which are mediators of inflammation (Kim et al., 2004).

According to Narinderpal et al. (2013), the arthritic syndrome was suppressed by withaferin A, and hydrocortisone-treated animals lost weight, whereas withaferin A-treated animals gained weight.

Extracts from *Ricinus communis* leaves and WS roots were given to arthritic rats. It was noted that WS extract reduced paw inflammation and arthritic score, hyperfunctioning of the thymus and spleen, and restored ALP levels. Histological studies demonstrated apparent healing of inflamed joints (Hussain et al., 2021). All these effects of WS extract indicate an improvement in bone quality, as per Nagareddy and Lakshmana (2006) and Mohammadi et al. (2022).

Antistress or adaptogen activity

The neurotransmitter gamma amino-butyric acid (GABA) has an inhibitory effect on the brain through the reduction of neuron activity and inhibition of potential action generation in nerve cells (32). There is evidence that WS extract may have GABAergic activity, suggesting a possible reason for its adaptogenic properties (Yin et al., 2013; Candelario et al., 2015; Sonar et al., 2019).

W. somnifera roots include steroids, which act as exogenous adrenocortical steroids and reduce the adrenocorticotropic hormone (ACTH) secretion and, consequently, endogenous steroid production (Mishra et al., 2000).

Archana and Namasivayam (1999) demonstrated that the treatment with 100 mg/kg of a suspension from WS in rats submitted to stress resulted in lower corticosterone levels. Furthermore, lower levels of cortisol and epinephrine were reported in equines submitted to various stress types (separation, loud noise, and strenuous exercise) and treated with root extract of WS for 21 days (Priyanka et al., 2020).

Studies with human patients also revealed lower cortisol levels due to the usage of 125 or 300 mg/day of WS root extract, indicating its antistress and anxiolytic effect (Salve et al., 2019).

Antimicrobial activity

Withanolides have antimicrobial action (Singh and Kumar, 1998) since WR root extract has an antibacterial effect against bacteria, including *Bacillus subtilis*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Escherichia coli* (Singh et al., 2011; El-Boshy et al., 2013; Kumari and Gupta, 2015) as well as against *Staphylococcus aureus* (Bokaeian and Saeidi, 2015).

Inhibition of *S. aureus* and *Enterococcus* spp. resistant against the utilized first-line antibiotics by WS leaf extract was demonstrated by Bisht and Rawat (2014). In an *in vitro* study, Dharajiya et al. (2014) noted the antimicrobial activity of stem extract of WS at 100 mg/mL against *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Aspergillus niger*, and *Trichoderma viride.*

W. somnifera root extract at 20 ml/L of water reduces the severity degree, mortality rate, and recovery period associated with *E. coli* infection and increases the humoral and cellular immune responses (Kumari et al., 2020) in broilers. Furthermore, *W. somnifera* inhibits the infectious bursal disease virus *in vitro* (Ganguly et al., 2020).

Hypoglycemic and hypolipidemic activities

Plants have been utilized for diabetes mellitus (DM) in Indian medicine for a long time (Udayakumar et al., 2009), with WS having hypoglycemic and hypocholesterolemic effects (Andalu and Radhika, 2000).

Using *W. somnifera* root extracts in diabetic rats resulted in hypoglycemic and hypolipidemic effects (Udayakumar et al., 2009; Sarangi et al., 2013), probably because of higher hepatic metabolism and improvement in insulin release from pancreatic β-cells (Navinder et al., 2013).

Gorelick et al. (2015), in a cultured cells study, verified that the WS leaf extract is more potent in increasing glucose uptake in skeletal muscle cells and adipocytes in comparison with the WS root extract. They also determined that withanolide A is responsible, at least partly, for the antidiabetic impact of the WS. A similar impact was reported by Shah et al. (2017) in cultured adipocytes, by Tupe et al. (2017) in *in vitro* studies, and by Mini et al. (2020) in rats.

Hyperlipidemia refers to elevated levels of lipids circulating in the blood (Mushtaq et al., 2016). Anwer et al. (2017) indicated lower levels of total cholesterol, its derivatives, and triglycerides in streptozotocin-induced type 2 diabetic rats treated with 200 or 400 mg/kg WS.

Uthirapathy and Tahir (2021) found that the hypolipidemic effect of WS may be the reduction in the HMG-CoA reductase activity. The same authors also mentioned that the impact of WS root extract on total cholesterol and LDL levels is comparable to that of standard medication atorvastatin in hyperlipidemic rats.

Effects of *W. somnifera* on broiler production

The beneficial effects of herbs result from the improvements in feed consumption, secretion of digestive enzymes, immune system stimulation, and antimicrobial, anthelmintic, anti-inflammatory, and antioxidant properties (Suganya et al., 2016) as well as the improvement of bone mineralization and strength (Tahmasbi et al., 2012; Mirakzehi et al., 2013). The effects of WS on growth rate and carcass traits are illustrated in Figure 3.

Productive performance

Generally, any stress factor may impair feed intake and, consequently, growth rate. Thus, using adaptogen plants as WS will enhance feed intake, as discussed by Mushtaq et al. (2011) in broilers fed WS extract at 10, 20, and 30 g/L of water. Vasanthakumar et al. (2015) also noted higher feed intake (3954 in the control group versus 4580 and 4423 g in experimental groups) in broilers fed diets with 1% of WS powder or 0.15% of WS extract, respectively. High feed intake in birds due to the WS administration was also reported by Sanjyal and Sapkota (2011), Pandey et al. (2013), Joshi et al. (2015), Kumar et al. (2018 a), and Nagar et al. (2020). On the contrary, some researchers pointed out that WS may not affect the feed intake of broilers (Kale et al., 2016; Azimi et al., 2020; Nagar et al., 2021).

Dietary WS has been associated with increased feed intake, stimulation of the thyroid gland (Sharma et al., 2018; Abdel-Wahhab et al., 2019) and consequently of poultry's metabolism, increased testosterone and estradiol secretion (Lopresti et al., 2019; Gopal et al., 2021), and increased muscle mass (Chikwa et al., 2018; Lande et al., 2019; Lee et al., 2020). In addition to the improvement of the metabolism rate, WR may raise the secretion of endogenous enzymes and enhance hepatic function and hepatic protein biosynthesis, which will be reflected in a higher growth rate (Salem et al., 2022).

Hence, an improvement in the growth rate is expected, as demonstrated by Joshi et al. (2015), Vasanthakumar et al. (2015), Kale et al. (2016), and Chikwa et al. (2018). However, some studies showed no effect on broilers' weight gain due to using WS extracts (Lande et al., 2019; Azimi et al., 2020; Nagar et al., 2020, 2021).

The feed conversion ratio (FCR) determines the profitability of the broiler industry. Ayurveda is gaining popularity in numerous countries, and it is stated that the WS can raise metabolism and stimulate digestion (Wal et al., 2014) and may enhance FCR as demonstrated by Saini et al. (2017). Moreover, Jyotsana et al. (2019) observed greater development of the intestinal villus in broilers receiving 0.5, 0.75, and 1% WR root powder in the diets. Raghavan et al. (2011) also reported an improvement in the activity of the digestive enzymes amylase, protease, and lipase in broilers, contributing to better nutrient absorption.

Consumption of WS results in better FCR in broilers at 0.15% root extract (Vasanthakumar et al., 2015), 0.25 and 0.5% root powder (Kale et al., 2016), and 100 mg/kg root powder (Kumar et al., 2018 a).

The results of FCR in broilers fed diets with WS are conflicting because some researchers have found no changes in this parameter (Joshi et al., 2015; Azimi et al., 2020; Nagar et al., 2021).

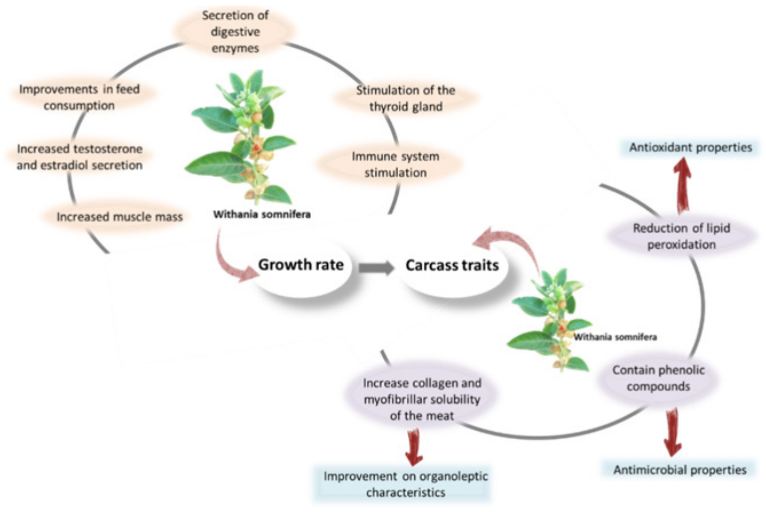

Figure 3. The effects of *Withania somnifera* on growth rate and carcass traits

Since dietary WS enhances productivity performance in broilers, a better net return would be expected (Javed et al., 2009; Sanjyal and Sapkota, 2011; Ansari et al., 2013; Kale et al., 2016).

Carcass traits and meat quality

Carcass traits

As previously shown, WS enhances feed intake, weight gain, and meat deposition and has an anabolic effect, which may improve broilers' carcass traits. The use of 0.5–2% WS powder in cockerels diets leads to higher values of carcass yield and relative weight of viscera compared to the control group (Maysa, 2023). Similar results were obtained by Singh et al. (2017) and Biswas et al. (2020). Conversely, dietary inclusion of WS at 0.75 or 1.5 g/kg WS dried leaves (Vasanthakumar et al., 2015), 50 or 100 mg/kg diet of WS extract (Kumar et al., 2018 a), or 250 mg/kg diet (Singh et al., 2020) showed no differences in the yield of the carcass, breast, thigh, and internal organs.

Meat quality

Meat quality evaluation is a key measure of meat palatability and acceptability to consumers (Singh et al., 2017). The use of herbs might enhance feed quality via their antioxidant and antibacterial properties associated with alkaloids, terpenoids, saponins, and phenolic compounds (Panchal and Singh, 2015). The antimicrobial activity contributes to food microbiological safety and quality. Antioxidant-rich herbs may protect against oxidative stress due to the reduction of lipid peroxidation (Orlowski et al., 2018).

From a quality point of view, the dietary intake of phytogenic compounds has been reported to have a beneficial impact on meat quality due to the antioxidant activities that reduce lipid oxidation (Mountzouris et al., 2009). The withaferin and withanolides present in the roots of *W. somnifera* are responsible for their various biological properties (Saini et al., 2017).

Rindhe et al. (2012) indicated an improvement in organoleptic characteristics of broiler meat, that is, appearance, odor, color, flavor, juiciness, texture, and palatability, due to the dietary inclusion of an herb mixture containing WS. They attributed this impact to the increase in collagen and myofibrillar solubility of the meat.

Udayakumar et al. (2009) reported hypocholesterolemic and hypolipidemic impacts of root or leaf extract of WS on the liver, heart, and kidney of diabetic rats. Singh et al. (2020 verified a reduction in total cholesterol content in broiler meat when the birds were fed diets containing 250 mg/kg WS, but no changes were observed in meat pH, cooking yield, water holding capacity, and contents of moisture, fat, protein, and ash due to WS.

On the contrary, Thakur et al. (2017) recorded a linear increase in crude protein content in the meat (86.84 to 90.1%) and a linear reduction in lipid content (7.5 to 4.33%) in the breast muscle of broilers fed ration without or with 0.5, 1, and 1.5% WS root powder.

Greene et al. (2021) fed broilers with diets containing an herbal mixture (*Ocimum sanctum*, *Withania somnifera*, and *Emblica officinalis*). They reported lower levels of free amino acids in breast meat as a result of the amino acid incorporation and use by the muscle for protein synthesis.

The oxidative stability of broiler meat may be enhanced by the hydroalcoholic extract of WS leaf, even when the birds were fed with oxidized oil in the diet, as demonstrated by Azimi et al. (2020). The MDA value was decreased from 0.297 (control group) to 0.208 (100 mg/kg WS) and 0.233 (200 mg/kg WS), indicating the antioxidant activity inhibitory effects on the lipid peroxidation due to the high flavonoid and phenolic contents (Senthil et al., 2015; Ganguly et al., 2018) of WS extracts.

Blood profile

Blood cells

Erythrocyte membranes contain unsaturated fatty acids and are more exposed to oxygen than other tissue, being more susceptible to oxidative damage, and hemoglobin is a strong catalyst that may begin lipid peroxidation (Asgary et al., 2005; Biswas et al., 2020). The hemoprotective effect of WS may be caused by the antioxidant activity, the stimulation of stem cell proliferation (Mishra et al., 2000), and the increase in bone marrow cellularity (Ansari et al., 2013). Ansari et al. (2013) demonstrated that WS is a rich source of iron (647 ppm), which may elevate the synthesis of hemoglobin (Hb) in broilers. Higher white and red blood cells count and Hb concentration as a result of using WS extract have been recorded by Chikwa et al. (2018), Biswas et al. (2020), Kumari et al. (2015), and Abdallah et al. (2016).

The stimulus to hematopoiesis was also reported by Tikore et al. (2019) in fenvalerate-treated broilers. The authors indicated that fenvalerate reduced Hb levels (11.83 mg/dL) and leukocyte counts (11.60 $10^3/mm^3$) that are associated with their lower production in the lymphoid tissues or their higher lysis caused by the fenvalerate in the bone marrow, but these values were restored by WS root powder supplementation at 200 mg/kg feed (Hb levels, 12.84 mg/dL; leukocytes 12.77 $10^3/mm^3$).

Chronic stress induces corticosterone secretion, resulting in heterophil release into the blood (Grzelak et al., 2017). Biswas et al. (2020) reported a higher heterophil-to-lymphocytes ratio in heat-stressed broilers (0.59). However, they noted that WS powder reduced this value (0.32) and increased lymphocytes level. This is probably due to the attenuating effect of the withanolides on the hypothalamic-pituitary-adrenal axis activity (Lopresti et al., 2019; Salve et al., 2019), which results in the inhibition of the ACTH secretion in the adrenal gland and norepinephrine from sympathetic nerve ends.

Tikore et al. (2019) also recorded an elevation in heterophil count (50%) and lower values of hematocrit (29.17%) and lymphocytes (41%) in cockerels treated with fenvalerone. However, an improvement in these values was observed due to the protective influence of WS root powder (45.67%, 36.33%, and 46.17%, respectively).

Blood enzymes

Excessive oxidation of lipids can change the physical properties of cellular membranes and can result in covalent modifications of proteins and nucleic acids (Gaschler and Stockwell, 2017). Improvements in the serum levels of lipid peroxidation, glutathione, glutathione peroxidase, and superoxide dismutase in broilers fed diets with 100 mg/kg WS root extract were reported by Vasanthakumar et al. (2015).

Hepatoprotection is determined by concentrations of the enzymes alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST).

The hepatoprotection by WS occurs by reducing the level of lipid peroxidation due to the presence of withanolides and flavonoids (Alam et al., 2011) having antioxidant properties (Fernando et al., 2013; Hassanin et al., 2020; Saggam et al., 2021). Reduction in blood levels of ALT, AST, and ALP in broilers was recorded by Ansari et al. (2013) and Kumari et al. (2015), indicating a possible hepatoprotective effect of WS root powder. However, Ahmed et al. (2015) showed an increased level of ALP in broilers and no changes in AST and ALT levels caused by WS, in comparison with the control group. The authors attributed these higher ALP levels to increasing osteoblastic activity.

Blood biochemical constituents

The reduction in the HMG-CoA reductase activity by the WS action (Sarangi et al., 2013; Acharya et al., 2020) or the high fiber (22–38.7%) and phytosterol content in WS root powder (Acharya et al., 2020) is responsible for the hypolipidemic effect. Moreover, the hypocholesterolemic impact may also be caused by the higher bile salts excretion in feces and higher biosynthesis rate, leading to higher levels of LDL reabsorption to refill the depleted hepatic cholesterol (Acharya et al., 2020). The enterohepatic circulation reveals low cholesterol pools in the liver and an alteration in lipoprotein metabolism (Visavadiya and Narasimhacharya, 2011).

Low levels of total cholesterol in the serum of broilers as a result of using 2.5 and 5% of WS root were described by Ansari et al. (2013). Kale et al. (2016) noted the hypolipidemic impact only on triglyceride levels in broilers fed diets with 0.25 and 0.5% WS root powder. However, Kumar et al. (2018 b) reported no effect on the levels of total protein, total cholesterol, and HDL in broilers. Ahmed et al. (2015) also indicated no differences in HDL and LDL levels in broilers fed 0.75 and 1.5 g/kg of WS dried leaves.

Most plasma proteins are synthesized and secreted by the liver, the most abundant being albumin (Schreiber, 1978). An indicator of hepatic damage is the lowering of serum total protein, albumin, and globulin concentrations and lower albumin/globulin ratio (Bharathi et al., 2011). Ansari et al. (2013) supplemented the broiler's diet with 1.25, 2.5, and 5% WS roots and indicated an increase in total serum proteins with 2.5 and 5% supplementation in comparison with the control group. However, no differences in serum protein were reported by Ahmed et al. (2015), Kale et al. (2016), Pedhavi et al. (2017), and Kumar et al. (2018 b).

W. somnifera has a beneficial effect even in broilers intoxicated or infected with a pathogen. Bharathi et al. (2011) emphasized that chlorpyrifos-intoxicated broilers were treated with 0.1% dietary WS for 13 days and showed higher total protein, albumin, and globulin values, suggesting a therapeutic potential of the herb.

Kumari et al. (2015) infected broilers with *Salmonella gallinarum*, causing degenerative changes in the liver, and reducing the protein concentrations in the blood. However, total protein, albumin, and globulin contents were higher in the group supplemented with 0.5% WS root powder. Similarly, Ganguly et al. (2020) also infected broilers with the infectious bursal disease virus. Improvements in total protein and albumin concentration, irrespective of virus infection, were observed when broilers received 1% WS root extract in the diet.

Concerning glucose levels, Mushtaq et al. (2011) supplemented the water with WS and reported low blood glucose levels in broilers receiving 10 g/L (86.16 mg/dL), 20 g/L (73.83 mg/dL), and 30 g/L (92.16 mg/dL) in comparison with the control group (104.16 mg/dL). However, several reports indicated no changes due to the use of WS (Ahmed et al., 2015; Pedhavi et al., 2017; Ganguly et al., 2020).

Immunity

Immune protective mechanisms comprise the elaboration of potent inflammatory molecules, antibodies, and killer cell activation, which together destroy invading microorganisms, pathogenic autoreactive cells, and tumors (Chattopadhyay and Cone, 2007). WS root and leaf extract can enhance innate immunity while increasing interferon-gamma and T cells CD3+ and CD4+ in humans, suggesting a major role in supporting innate and adaptative immunity (Tharakan et al., 2021).

According to Malik et al. (2007) and Tharakan et al. (2021), WS is a plant rich in withanolide A that is highly efficient in improving immune response through increasing T and B cell proliferation and enhancing macrophage activation and immunoglobulin secretion.

The impacts of WS on the immune function of broiler chickens are illustrated in Figure 4.

Lymphoid organs

The development status of the thymus, bursa of Fabricius, and spleen may affect the immune function and disease resistance ability (Fan et al., 2013).

The effects of WS on viral load and cell-mediated immunity in chicks were evaluated by Latheef et al. (2017). The authors found that the supplementation of 1% WS in the diet was sufficient to increase CD4+ and CD8+ T cell count and reduce viral load in the thymus, spleen, and liver in chicks challenged with infectious anemia virus, an immunosuppressor virus, comparable to the uninfected group, revealing the immunomodulatory effects of WS.

Improvements in the weight of the thymus, spleen, and bursa were reported by Ansari et al. (2013) when feeding broilers with diets containing 1.25, 2.5, and 5% WS. However, Kumar et al. (2018 b) have not seen any differences in spleen weight as a result of using 50 and 100 mg/kg WS in diets for broilers. On the contrary, Azimi et al. (2020) evaluated the impact of diets containing oxidized oil, with or without WS leaf extract, on lymphoid organs in broilers at 42 days of age. They found that only the spleen weight was elevated due to the WS dietary inclusion at 100 and 200 mg/kg. Furthermore, no effect was observed on the weight of the thymus and bursa.

Antibody titers (AT)

Birds receiving 10, 20, and 30 g WS/L water presented increased AT against infectious bronchitis and infectious bursal disease. However, no differences were reported in AT against Newcastle disease. Moreover, Kumar et al. (2018 b) did not find any differences in AT against Newcastle disease in broilers receiving 50 and 100 mg/kg WS root extract. However, Ansari et al. (2013) reported high levels of AT against Newcastle disease in broilers fed diets with 1.25, 2.5, and 5% WS roots.

Higher AT against Ranikhet disease was demonstrated by Vasanthakumar et al. (2015) in broilers receiving diets containing 1% WS root powder (7.3 $\log_2$) or 0.15% WS leaf extract (7 $\log_2$), in comparison with the control birds (6.6 $\log_2$).

Recent knowledge and future prospects

The current knowledge about *Withania somnifera* indicates that the inclusion of WS in broiler diets may improve the following:

– Feed intake, weight gain, and feed conversion ratio (FCR) due to its effects on the metabolism rate and digestion stimulation,

– Meat quality because of its antioxidant property, reducing lipid peroxidation. In addition, WS has hypocholesterolemic and hypolipidemic properties that come against the consumers' concerns,

– Hematopoiesis, probably due to the inhibition of ACTH secretion and reduction of glucocorticoid release, mainly during chronic stress situations,

– Biochemical blood constituents, reducing the LDL and glucose levels and increasing total protein levels,

– Immune status due to the stimulus to lymphoid organs, the proliferation of T and B cells, macrophage activation, and immunoglobulin secretion.

Thus, *Withania somnifera* may be utilized as a growth promoter in broiler farming to enhance productivity, meat quality, blood profile, and immune status under conventional and organic farming.

Conclusion

The root extract of *Withania somnifera* is a promising phytoherb for poultry production as it can enhance production, meat quality, skeletal system, blood profile, and immune status of the birds. However, further research is needed to determine the optimal dietary inclusion level and the action mode of the plant.

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Author contributions

All authors contributed equally to this work. They have read and agreed to the published version of the manuscript.

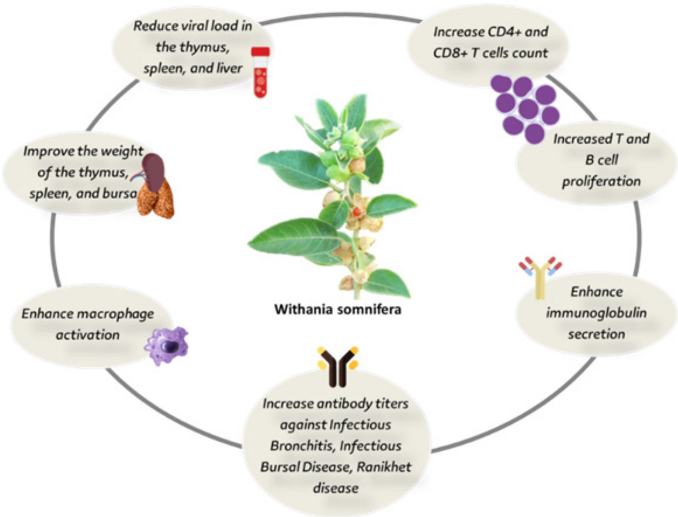

Figure 4. The impacts of *Withania somnifera* on immune function of broiler chickens

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Conflicts of interest

The authors declare no conflicts of interest.

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