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Dietary supplementation of amla (*Emblica officinalis*) and Shatavari (*Asparagus racemosus*) improves haematological profile and alleviates stress in broiler chickens

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Abstract
The present study was conducted to evaluate the effects of dietary supplementation of Amla (*Emblica officinalis*) and Shatavari (*Asparagus racemosus*) on the haematological profile and stress indicators of broiler chickens. A total of 288-day-old Cobb broiler chicks were randomly allocated to eight dietary treatments with four replicates of nine birds each under a completely randomized design and reared for 42 days. The control group received a basal diet, whereas the remaining groups were supplemented with different levels and combinations of Shatavari root powder and Amla powder. Blood samples were collected on the 42nd day of the feeding trial and analysed for total erythrocyte count (TEC), total leukocyte count (TLC), haemoglobin concentration (Hb), heterophil percentage, lymphocyte percentage and heterophil-to-lymphocyte (H/L) ratio. Dietary supplementation significantly ($P<0.05$) influenced all the haematological parameters studied. The highest TEC ($2.623 \times 10^6/\mu L$) and haemoglobin concentration (10.055 g/dL) were recorded in birds receiving 0.50% Shatavari supplementation (T_1). Heterophil percentage declined from 29.225% in the control group to a minimum value of 23.440% in birds receiving combined phytogenic supplementation, whereas lymphocyte percentage significantly ($P<0.05$) increased in the combined supplementation groups (66.71-67.18%) compared with the control group (62.29%). The H/L ratio, a reliable indicator of chronic stress, was markedly reduced from 0.469 in the control group to 0.348 in birds receiving combined phytogenic supplementation. Combined phytogenic supplementation improved stress resilience, as evidenced by reduced heterophil-to-lymphocyte ratios and enhanced lymphocyte percentages, thereby supporting the use of Amla and Shatavari as natural feed additives for improving bird welfare and promoting sustainable poultry production.

Keywords: Broiler chickens, *Emblica officinalis, Asparagus racemosus,* haematological profile, heterophil-to-lymphocyte ratio, phytogenic feed additives, stress indicators

Introduction
The poultry sector represents one of the most dynamic segments of the global livestock industry and contributes substantially to food and nutritional security by providing affordable, high-quality animal protein. In recent decades, rapid intensification of broiler production has enabled producers to meet the increasing consumer demand for poultry meat. However, intensive production systems frequently expose birds to a variety of environmental, nutritional and managerial stressors, including high stocking density, vaccination procedures, transportation, feed transitions, handling practices and fluctuations in ambient temperature. These stressors adversely affect physiological homeostasis, immune competence, growth performance and overall welfare of broiler chickens (Lara and Rostagno, 2013; Scanes, 2016). Stress in poultry is characterized by a cascade of neuroendocrine responses involving activation of the hypothalamic-pituitary-adrenal (HPA) axis and subsequent release of corticosterone, the principal glucocorticoid hormone in birds. Prolonged exposure to stress has been associated with reduced feed efficiency, impaired immune responses, increased susceptibility to diseases and alterations in haematological characteristics (Maxwell, 1993; Scanes, 2016) [16, 24, 17]. Consequently, minimizing stress and improving physiological adaptability have become major objectives of modern poultry nutrition and management strategies.

For several decades, antibiotic growth promoters (AGPs) were routinely incorporated into poultry diets to improve growth performance and maintain flock health. Although AGPs effectively enhanced feed utilization and productivity, concerns regarding the emergence of antimicrobial resistance and the presence of drug residues in poultry products led to restrictions and bans on their use in many countries (Castanon, 2007). The withdrawal of AGPs has stimulated extensive research into natural alternatives capable of promoting productivity while safeguarding public health.

Among the proposed alternatives, phytogenic feed additives have emerged as one of the most promising nutritional strategies in poultry production. Phytogenic feed additives include herbs, spices, essential oils and plant-derived extracts rich in biologically active compounds such as polyphenols, flavonoids, alkaloids, tannins and saponins. These compounds have been reported to improve digestive efficiency, modulate intestinal microflora, enhance immune responses and exert antioxidant and anti-inflammatory activities (Greathead, 2003; Windisch *et al.*, 2008). Windisch *et al.* (2008) emphasized that phytogenic products may serve as effective substitutes for conventional antibiotic growth promoters because of their multifunctional biological properties and consumer acceptability [6, 9, 26].

Recent reviews have further highlighted the role of phytogenic feed additives in improving gastrointestinal health, production efficiency and systemic resilience in poultry birds. In addition to enhancing performance traits, phytogenic compounds may alleviate oxidative stress and strengthen host defense mechanisms, thereby improving bird welfare under commercial production conditions (Abdelli *et al.*, 2021) [1].

Among the physiological indicators used to evaluate stress in poultry, the heterophil-to-lymphocyte (H/L) ratio has received considerable attention because of its reliability and ease of determination. Gross and Siegel (1983) [10] demonstrated that the H/L ratio was a more reliable indicator of chronic stress than plasma corticosterone measurements under several experimental conditions, thereby establishing its importance in poultry stress research.

Among the medicinal plants explored as phytogenic feed additives, Amla (*Emblica officinalis*), commonly known as Indian gooseberry, has attracted considerable scientific interest because of its diverse pharmacological properties and high nutritional value. Amla fruit is one of the richest natural sources of vitamin C and contains a wide range of bioactive constituents including emblicanin A and B, gallic acid, ellagic acid, tannins, flavonoids and polyphenolic compounds (Scartezzini and Speroni, 2000) [25]. These phytochemicals are recognized for their potent antioxidant activity and their ability to neutralize reactive oxygen species generated during physiological stress. In addition to its antioxidant potential, Amla exhibits antimicrobial, hepatoprotective, hypolipidemic, anti-inflammatory and immunomodulatory activities, making it an attractive natural feed additive in poultry nutrition (Krishnaveni and Mirunalini, 2010) [13].

Several investigations have demonstrated the beneficial effects of Amla supplementation in poultry diets. Dietary inclusion of Amla has been associated with improved growth performance, enhanced antioxidant status and better immune responsiveness in broiler chickens (Patel *et al.*, 2015) [21]. The protective effect of Amla has largely been attributed to its capacity to reduce oxidative damage, preserve cellular integrity and enhance the activity of endogenous antioxidant systems. Moreover, the high vitamin C content of Amla may play a crucial role in minimizing the adverse effects of stress by modulating corticosterone secretion and supporting immune function.

Another medicinal herb of considerable importance in Ayurvedic medicine is Shatavari (*Asparagus racemosus*), which has traditionally been classified as a "Rasayana" herb due to its rejuvenating and restorative properties. The roots of Shatavari contain steroidal saponins collectively known as shatavarins, along with flavonoids, alkaloids, glycosides and mucilage (Rege *et al.*, 1989) [22]. These compounds possess antioxidant, anti-inflammatory, adaptogenic and immunostimulatory activities. Adaptogens are natural substances that enhance the ability of an organism to resist physical, chemical and biological stressors while maintaining physiological homeostasis (Panossian and Wikman, 2010) [20].

Experimental studies have demonstrated that Shatavari supplementation improves immune responses, enhances disease resistance and supports physiological adaptation under stressful conditions (Kamat *et al.*, 2000). Kumari *et al.* (2012) reported that supplementation of *Asparagus racemosus* improved physiological responses and health status in poultry birds. Rekhate *et al.* (2010) [12, 15, 23] reported favourable effects of Shatavari supplementation on productive performance and health status in poultry birds. The adaptogenic activity of Shatavari is believed to involve modulation of neuroendocrine responses, stabilization of cellular membranes and improvement of antioxidant defense mechanisms. These characteristics suggest that Shatavari may be particularly useful in alleviating stress-induced physiological disturbances commonly encountered under intensive broiler production systems.

Considering their complementary biological activities, the combined use of Amla and Shatavari may provide synergistic benefits in poultry nutrition. While Amla primarily contributes through its antioxidant and immunomodulatory actions, Shatavari exerts potent adaptogenic and anti-stress effects. Therefore, simultaneous dietary supplementation of these herbs has the potential to improve physiological resilience, maintain immune competence and enhance the ability of birds to cope with stress.

Haematological parameters are among the most sensitive indicators of health status and physiological adaptation in birds. Total erythrocyte count (TEC) and haemoglobin concentration reflect oxygen transport capacity and indirectly indicate the metabolic and nutritional status of broilers. Similarly, total leukocyte count (TLC) provides valuable information regarding immune competence and host defense mechanisms (Jain, 1986; Campbell, 2015). Differential leukocyte counts, particularly heterophil and lymphocyte percentages, are widely used to assess the magnitude of stress responses in poultry. An increase in heterophil count accompanied by a decline in lymphocyte count generally reflects stress-induced immunological alterations mediated through elevated corticosterone secretion (Maxwell, 1993; Aengwanich, 2008) [11, 5, 2].

Although numerous studies have independently evaluated the effects of phytogenic feed additives in poultry, scientific information regarding the combined influence of Amla and Shatavari on haematological characteristics and stress indicators remains limited. Most previous investigations have focused primarily on growth performance, carcass characteristics or feed efficiency, with comparatively little

attention being paid to blood profiles and physiological markers of stress. Therefore, the present investigation was undertaken to evaluate the effect of dietary supplementation of different levels of *E. officinalis* and *A. racemosus* on total erythrocyte count, total leukocyte count, haemoglobin concentration, heterophil percentage, lymphocyte percentage and heterophil-to-lymphocyte ratio in broiler chickens. It was hypothesized that the combined supplementation of these phytogenic additives would improve haematological status and alleviate physiological stress, thereby supporting their use as natural alternatives for promoting health and welfare in commercial broiler production systems.

Materials and Methods

Experimental birds and management: The experiment was conducted at the Poultry Unit, Department of Animal Husbandry and Dairying, Chandra Shekhar Azad University of Agriculture and Technology, Kanpur, Uttar Pradesh, India. A total of 288-day-old commercial Cobb broiler chicks were procured from a single hatch and randomly allocated to eight dietary treatment groups following a completely randomized design (CRD). Each treatment consisted of four replicates with nine chicks per replicate, resulting in 36 birds per treatment. The experimental trial lasted for 42 days.

Birds were reared under standard management practices throughout the experimental period. Feed and clean drinking water were provided ad libitum. Routine vaccination and health care practices were followed as per the university poultry management schedule. All birds were maintained under similar environmental and hygienic conditions.

Experimental design and dietary treatments

The birds were randomly assigned to eight dietary treatments designated as T_0 to T_7 under a completely randomized design. The control group (T_0) received the basal diet without supplementation. Birds in treatment T_1 received the basal diet supplemented with 0.50% Shatavari root powder, whereas those in T_2 received the basal diet supplemented with 0.75% Shatavari root powder. Birds in treatment T_3 were fed the basal diet supplemented with 0.50% Amla powder, while those in T_4 received the basal diet supplemented with 0.75% Amla powder. The combination treatment groups included T_5, which received the basal diet supplemented with 0.50% Shatavari root powder and 0.50% Amla powder; T_6, which received the basal diet supplemented with 0.75% Shatavari root powder and 0.50% Amla powder; and T_7, which received the basal diet supplemented with 0.50% Shatavari root powder and 0.75% Amla powder. The basal diets were formulated to meet or exceed the nutrient requirements recommended by the Bureau of Indian Standards (BIS) for broiler chickens.

Blood sample collection: At the end of the six-week feeding trial (42nd day), blood samples were collected from representative birds of each replicate for haematological analysis. Approximately 2 mL of blood was collected aseptically into sterilized vials containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. The collected samples were immediately transported to the laboratory for further analysis.

Haematological analysis: The haematological parameters evaluated included total erythrocyte count (TEC), total leukocyte count (TLC), haemoglobin concentration (Hb), heterophil percentage, lymphocyte percentage and heterophil-to-lymphocyte (H/L) ratio.

Total erythrocyte count was determined using a Neubauer haemocytometer according to the method described by Natt and Herrick (1952) [19]. Blood samples were diluted with Vallarino's solution at an appropriate dilution and erythrocytes were counted microscopically. The results were expressed as $\times 10^6$ cells/µL.

Total leukocyte count was estimated using a Neubauer counting chamber following the procedure described by Jain (1986). Thomas diluting fluid was used for blood dilution and leukocytes were counted under a microscope. The values were expressed as $\times 10^3$ cells/µL.

Haemoglobin concentration was estimated spectrophotometrically at 540 nm using the cyanmethemoglobin method with Drabkin's reagent as described by Fudge (2000). Haemoglobin values were expressed in g/dL.

Differential leukocyte counts (DLC) were determined using an automated haematology analyser (Beckman Coulter R AC T_5 Diff AL), and heterophil and lymphocyte percentages were recorded. The heterophil-to-lymphocyte ratio was calculated by dividing the heterophil percentage by the lymphocyte percentage.

Statistical analysis

The experimental data were analysed statistically using a completely randomized design (CRD) with analysis of variance (ANOVA) based on the F-test as described by Snedecor and Cochran (1994). Differences among treatment means were considered significant at *P* < 0.05 and are represented by different superscripts within each column.

Results and Discussion

Effect of dietary supplementation of Amla and Shatavari on haematological parameters of broiler chickens

The effects of dietary supplementation of Amla powder (AP) and Shatavari root powder (SRP) on the haematological profile of broiler chickens are presented in Table 1. The results demonstrated that supplementation with these phytogenic additives significantly (*P<0.05*) influenced total erythrocyte count (TEC), total leukocyte count (TLC), haemoglobin concentration (Hb), heterophil percentage, lymphocyte percentage and heterophil-to-lymphocyte (H/L) ratio.

Table 1: Effect of dietary supplementation of Amla and Shatavari on haematological parameters of broiler chickens at 42 days of age

Treatments	TEC ($\times 10^6$/µL)	TLC ($\times 10^3$/µL)	Hb (g/dL)	Heterophil (%)	Lymphocyte (%)	H/L Ratio
T_0 (Control)	2.615^{a}	258.88^{ab}	9.925^{c}	29.225^{a}	62.29^{d}	0.469^{a}
T_1	2.623^{a}	257.96^{b}	10.055^{a}	26.680^{b}	63.89^{c}	0.417^{b}
T_2	2.595^{a}	244.42^{e}	10.000^{abc}	26.218^{b}	64.96^{b}	0.403^{c}
T_3	2.468^{bc}	260.95^{a}	9.928^{c}	26.400^{b}	65.29^{b}	0.404^{bc}
T_4	2.393^{e}	250.79^{c}	10.013^{ab}	26.253^{b}	65.38^{b}	0.401^{c}
T_5	2.438^{d}	247.06^{d}	9.978^{abc}	24.280^{c}	66.71^{a}	0.361^{d}
T_6	2.495^{b}	244.67^{e}	9.935^{bc}	24.433^{c}	67.11^{a}	0.348^{e}
T_7	2.453^{cd}	246.90^{d}	9.990^{abc}	23.440^{c}	67.18^{a}	0.354^{e}

Means bearing different superscripts within a column differ significantly at P<0.05.

Total erythrocyte count (TEC)

The TEC values varied significantly ($P<0.05$) among treatment groups and ranged from 2.393 $\times 10^6$/µL (T_4) to 2.623 $\times 10^6$/µL (T_1). Birds supplemented with lower levels of SRP exhibited TEC values comparable to the control group, whereas combination treatments maintained erythrocyte counts within the physiological range.

Red blood cells are responsible for oxygen transport and serve as indicators of metabolic competence and haematopoietic efficiency. The maintenance of normal TEC values observed in the present study suggests that dietary supplementation of Amla and Shatavari did not adversely affect erythropoiesis. The antioxidant constituents of Amla, particularly vitamin C, emblicanins and polyphenolic compounds, are known to protect erythrocyte membranes against oxidative injury and prolong cellular integrity (Krishnaveni and Mirunalini, 2010). Similarly, the steroidal saponins and flavonoids present in Shatavari have been reported to support physiological homeostasis and normal blood cell production (Rege *et al.*, 1989) [22, 13].

The present findings are consistent with Mushtaq *et al.* (2012) [18], who observed improved erythrocytic indices in broilers receiving phytogenic feed additives. Likewise, Borkar *et al.* (2002) [4] reported a significant increase in TEC values following supplementation with the herbal immunomodulator "Growell" in vaccinated chicks. However, Alkassie (2009) [3] reported non-significant effects of peppermint supplementation on erythrocyte profiles, indicating that the response may vary according to plant species and dosage.

Total leukocyte count (TLC)

Dietary treatments significantly affected TLC values, which ranged from 244.42 $\times 10^3$/µL in T_2 to 260.95 $\times 10^3$/µL in T_3. Despite these differences, all values remained within physiological limits, indicating that supplementation did not compromise immune stability.

Leukocytes are fundamental components of host defense and their numbers often reflect immunological status. The maintenance of optimal leukocyte populations in supplemented groups may be attributed to the immunomodulatory properties of both herbs. Amla has been shown to stimulate immune function through enhancement of antioxidant defenses and modulation of cytokine responses (Patel *et al.*, 2015), whereas Shatavari has been recognized for its immunostimulatory potential and ability to enhance resistance to stress and infections (Kamat *et al.*, 2000) [12].

Comparable findings have been reported by Mushtaq *et al.* (2012) [18], who observed improved leukocyte profiles in birds fed phytogenic additives. Similarly, Borkar *et al.* (2002) documented enhanced TLC values in chicks receiving herbal preparations. In contrast, Alkassie (2009) observed no significant effect of herbal supplementation on leukocyte counts [21, 4, 3].

Haemoglobin concentration (Hb)

Haemoglobin concentration differed significantly ($P<0.05$) among treatments and ranged from 9.925 g/dL in the control group to 10.055 g/dL in T_1. Birds receiving SRP supplementation exhibited relatively higher haemoglobin concentrations compared with control birds.

Haemoglobin concentration is an important indicator of oxygen-carrying capacity and reflects the nutritional and physiological status of birds. The higher Hb concentrations observed in supplemented groups may be associated with improved nutrient utilization and reduced oxidative degradation of erythrocytes. The antioxidant activity of Amla may contribute to stabilization of erythrocyte membranes and maintenance of haemoglobin synthesis (Krishnaveni and Mirunalini, 2010). Similarly, adaptogenic compounds present in Shatavari may improve metabolic efficiency under stressful conditions.

These findings corroborate the observations of Mushtaq *et al.* (2012), who reported elevated Hb concentrations in broilers supplemented with phytogenic additives. Similar improvements in haematological traits have also been reported by Borkar *et al.* (2002). Conversely, Alkassie (2009) found no significant changes in Hb levels following herbal supplementation [4, 13, 18].

Heterophil percentage

The lowest heterophil percentages were observed in the combined supplementation groups, particularly T_6 and T_7. Birds receiving combined supplementation exhibited lower heterophil counts than unsupplemented birds.

Heterophils are the first line of cellular defense against infectious agents; however, elevated heterophil counts are commonly associated with stress-induced corticosterone secretion. Chronic stress stimulates redistribution of leukocytes, resulting in heterophilia and suppression of lymphocyte populations (Maxwell, 1993) [17]. Therefore, the reduction in heterophil percentages observed in supplemented groups suggests an alleviation of physiological stress.

The antioxidant activity of Amla and adaptogenic effects of Shatavari may have attenuated stress-mediated endocrine responses, thereby reducing heterophil mobilization. Similar reductions in heterophil percentages following anti-stress supplementation have been reported by Kumar *et al.* (2014) [14].

Lymphocyte percentage

Lymphocyte percentages increased significantly ($P<0.05$) with dietary supplementation, ranging from 62.29% in the control group to 67.18% in T_7. Combination treatments (T_5-T_7) exhibited significantly higher lymphocyte percentages.

Lymphocytes are central to adaptive immunity and their abundance reflects immunocompetence and physiological well-being. Under stressful conditions, elevated corticosterone levels induce lymphopenia, resulting in impaired immune responses (Gross and Siegel, 1983). Therefore, the increase in lymphocyte populations observed in supplemented birds indicates enhanced immune status and improved stress adaptation.

The immunomodulatory properties of Amla and Shatavari may have contributed to improved lymphocyte proliferation and survival. Similar findings have been documented in studies evaluating herbal immunostimulants in poultry (Dhama *et al.*, 2015) [7].

The observed alterations in heterophil and lymphocyte percentages in response to dietary supplementation of Amla and Shatavari are presented in Figure 1.

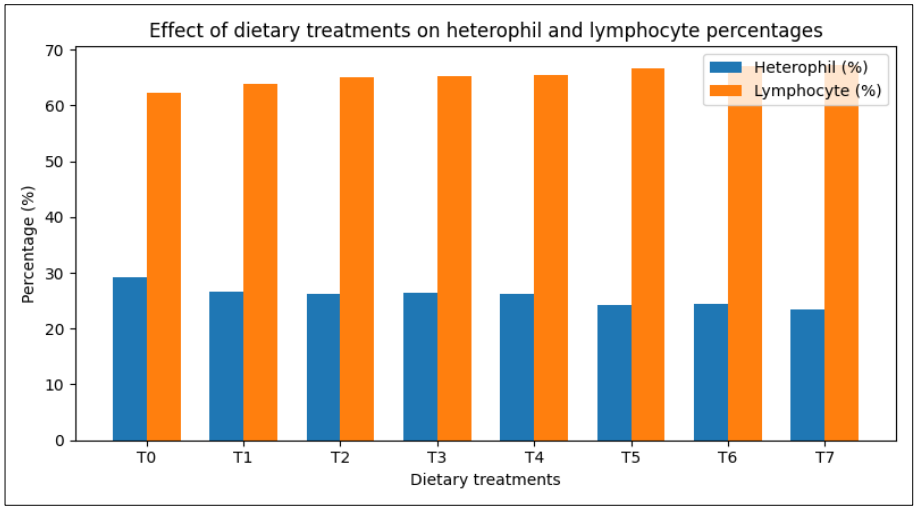

Fig 1: Effect of dietary supplementation of Amla and Shatavari on heterophil and lymphocyte percentages in broiler chickens on the 42nd day of the feeding trial.

Heterophil-to-lymphocyte (H/L) ratio

The H/L ratio was significantly reduced ($P<0.05$) by dietary supplementation. The highest H/L ratio was recorded in the control group (0.469), whereas the lowest ratio was observed in T_6 (0.348), followed by T_7 (0.354).

The H/L ratio is considered one of the most reliable indicators of chronic stress in poultry. Gross and Siegel (1983) [10] demonstrated that H/L ratio provides a practical and sensitive assessment of stress responses in birds, while Maxwell (1993) [17] further established its usefulness under commercial production systems. Elevated H/L ratios indicate stress-induced immunological disturbances, whereas lower ratios reflect improved welfare and adaptive capacity.

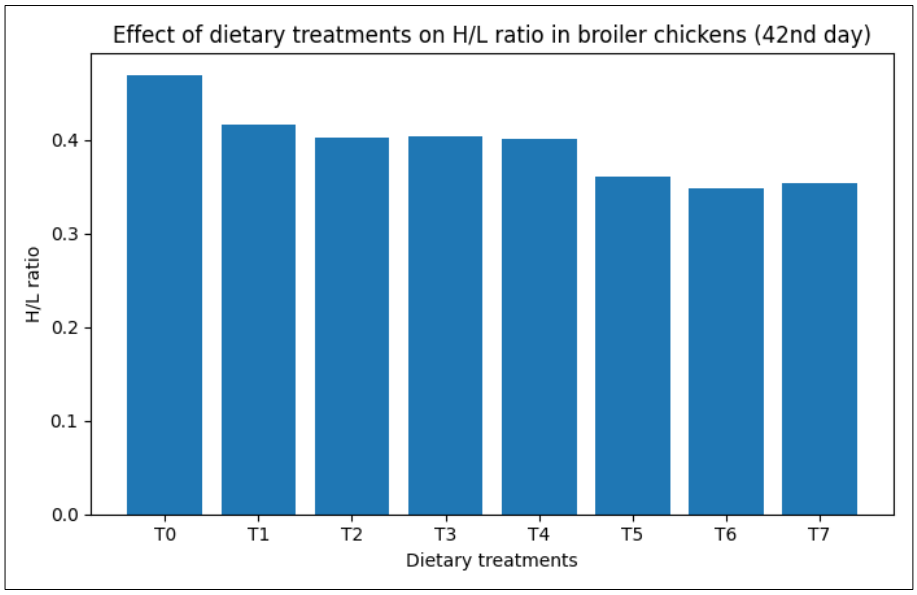

Fig 2: The influence of dietary treatments on the heterophil-to-lymphocyte (H/L) ratio, an established indicator of physiological stress in poultry

The marked reduction in H/L ratios observed in the present investigation clearly demonstrates the anti-stress potential of combined Amla and Shatavari supplementation. The antioxidant constituents of Amla may reduce oxidative stress and corticosterone-mediated cellular damage, while the adaptogenic properties of Shatavari may enhance resistance to environmental and physiological stressors (Panossian and Wikman, 2010). Kumar *et al.* (2014) similarly reported significant reductions in H/L ratio following supplementation with anti-stress agents in broilers. More recently, phytogenic feed additives have been recognized as effective nutritional tools for improving stress resilience and immune stability in poultry production systems (Abdelli *et al.*, 2021) [20, 14, 1].

Overall, the present findings indicate that dietary supplementation of Amla and Shatavari positively modulated haematological characteristics and alleviated physiological stress in broiler chickens. The combined supplementation groups, particularly T_6 and T_7, exhibited superior stress adaptation as evidenced by lower heterophil percentages and reduced H/L ratios. These results support the use of these phytogenic additives as natural anti-stress agents in commercial broiler production.

Conclusion: The present study demonstrated that dietary supplementation of Amla (*E. officinalis*) and Shatavari (*A. racemosus*) significantly influenced the haematological profile and stress indicators of broiler chickens.

Supplementation with these phytogenic feed additives maintained normal erythrocytic and leukocytic indices, indicating that their inclusion in broiler diets did not exert any adverse effect on blood health.

Broilers receiving supplemented diets exhibited improved haemoglobin concentrations and favourable modulation of leukocyte populations. The reduction in heterophil percentage, accompanied by a significant increase in lymphocyte percentage, resulted in lower heterophil-to-lymphocyte ratios in the supplemented groups compared to the control birds. Since the H/L ratio is a well-established indicator of chronic stress in poultry, these findings suggest that Amla and Shatavari supplementation effectively alleviated physiological stress and enhanced adaptive capacity in broiler chickens.

Among the dietary treatments, the combination groups, particularly T_6 and T_7, demonstrated superior anti-stress responses as evidenced by lower H/L ratios and improved lymphocyte percentages. The beneficial effects observed in the present investigation may be attributed to the antioxidant, immunomodulatory and adaptogenic properties of the bioactive constituents present in Amla and Shatavari.

Therefore, it may be concluded that dietary supplementation of Amla and Shatavari can be used as a safe and effective phytogenic strategy to improve haematological status and mitigate stress in broiler chickens. The findings support their potential application as natural alternatives to synthetic growth promoters for promoting bird welfare and sustaining productivity under commercial poultry production systems.

Practical Implications: The results of the present study have important implications for commercial poultry production. The ability of Amla and Shatavari supplementation to improve stress resilience without adversely affecting haematological health suggests that these medicinal herbs may be incorporated into broiler diets as natural anti-stress feed additives. Their use may be particularly beneficial during periods of environmental or management-related stress, such as heat stress, vaccination, transportation and high stocking density. In addition to improving bird welfare, phytogenic supplementation may contribute to reducing dependence on antibiotic growth promoters and support consumer demand for safer poultry products.

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