

*Original Research***Effect of Amla Extract Supplementation on Growth Parameters of *Escherichia coli* Infected Broiler Chickens**Adya Prakash Rath^{1*}, K. K. Jakhar², Renu Singh³, Sushma⁴ and Vikas Nehra⁵¹Department of Veterinary Pathology, GADVASU, Ludhiana, Punjab, INDIA^{2, 4&5}Department of Veterinary Pathology, LUVAS, Hisar, Haryana, INDIA³Department of Veterinary Pathology, DUVASU, Mathura, Uttar Pradesh, INDIA*Corresponding author: rathadya10@gmail.com

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Abstract

The present study was undertaken in broiler chickens to study the effect of dry fruit extract of amla supplementation on clinical signs, body weight and reduction in mortality in relation to the severity of colibacillosis. One hundred and sixty-eight day old healthy broiler chicks were divided into two groups (A and B) containing eighty-four birds each. Diet of all the chicks of group A was supplemented with grinded dry fruit extract of Amla @ 10g/kg of feed whereas, chicks of group B were given normal feed and water. At the age of 7 days chicks of group A1 and B1 were injected intraperitoneally with standard infective dose of pathogenic *E. coli* O120 to produce experimental colibacillosis. Clinical signs of *E. coli* infection were dullness, depression, inappetance, drooping of head and neck, closing of eyes, ruffled feathers, huddling together near heat source, hock sitting position and some of the infected birds exhibited respiratory distress and bloody diarrhoea. Clinical signs were almost similar to those observed in group B1 but the severity of the clinical signs was of lesser intensity. Mortality in non-supplemented infected group started from 24 hours post infection and overall mortality was 58.20 per cent. On the other hand, mortality in amla supplemented infected group started from 2 days post infection and overall mortality was 43.75 per cent. Hence, dry fruit extract of amla supplementation leads to reduction in mortality and severity of disease in *E. coli* infected broiler chicken.

Key words: Amla, *Escherichia coli* Infection, Growth Parameters, Mortality

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Introduction

Indian poultry industry has emerged, as an agro-based industry. Broiler production is the dynamic as well as the most rapidly expanding segment of the poultry industry in the country. Poultry today not only acts as income stabilizer but also provides regular and timely income as compared to crop and other livestock



farming. Poultry plays an important role in Indian diet as egg and chicken meat are rich sources of protein, vitamins and minerals. As per 19th livestock census 2012, total poultry population in India is 729.2 million and has a growth rate of 12.39% as compared to that of 2007 (Annual report, DAHD, 2016-17). India has become third largest egg producer in the world with a production of around 82.93 billion eggs during 2015-16 and fourth largest broiler producer after China, Brazil and USA. India had evolved as the world's 2nd largest poultry market with an annual growth rate of more than 14 per cent, producing 61 million tonnes or 3.6 per cent of global egg production (4th International poultry and livestock expo-2015). The annual growth rate of egg production is 5-8per cent. The poultry meat production is estimated to be 3.26 million tonnes (Annual report, DAHD, 2016-17).

Despite of front-runner in World's market Indian poultry industry faces a tremendous loss in the form of reduction in production and growth. Amongst these gastrointestinal infections ranges from insignificant economic effects to those that are severe and cause devastating losses (Mettifogo *et al.*, 2014). Although the disease can affect all age groups but it predominantly affects young ones with increased susceptibility to other diseases, decreased feed conversion efficiency and prolonged marketability (Barnes and Guy, 2003; Saif, 2008). Amongst these, the conditions affecting the gastro-intestinal tract are quite common and include salmonellosis, colibacillosis, ranikhet disease, coccidiosis, necrotic enteritis etc. Importance of these diseases can be judged from the fact that incidence of coccidiosis was found to be 15.5 % followed by *Escherichia coli* infections (14%), fowl typhoid, fatty liver syndrome and Ranikhet disease (Suresh *et al.*, 1990). Avian colibacillosis is a bacterial disease of birds caused by *E. coli*, which is considered as one of the principal causes of morbidity and mortality, associated with heavy economic losses to the poultry industry as it is associated with various disease conditions, either as primary pathogen or as a secondary pathogen (Lutful Kabir, 2010). *E. coli* has the ability to invade bloodstream leading to several internal infections like pericardium (pericarditis), liver (perihepatitis), peritoneum (peritonitis) and oviduct (salpingitis), colisepticemia, coligranuloma, synovitis etc. (Vandemaele *et al.*, 2002). *E. coli* turns pathogenic under adverse conditions of poor ventilation, overcrowding, immunosuppression etc (Goswami *et al.*, 2004).

Rural population of India normally uses medicinal plants for the treatment of some common infections of livestock and poultry. The medicinal properties of *Emblica officinalis* that is also commonly called as "Indian gooseberry" or "Amla" can be traced back in the ancient medical treatise like Ayurveda. Amla preparations increased the body weight gain and feed conversion efficiency (Singh *et al.*, 2010) in broilers. These preparations decrease the mortality rates and the cost of feed has been found to be decreased from 6.2 per cent to 13.5 per cent. Preparations reduce the fat accumulation increased dressing percentage, liver weight, spleen weight and whole gible weights (Mode *et al.*, 2009). Literature also revealed that dietary addition of *E. officinalis* (Amla) fruit powder had a positive effect on growth performance in commercial

broiler chickens (Patel *et al.*, 2016). Keeping in view the above facts, the present study will be undertaken broiler chickens to study the effect of dry fruit extract of amla supplementation on mortality, clinical signs and growth parameters.

Material and Methods

The approval for conducting the experiment was taken from the Institutional Animal Ethics Committee (IAEC), Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar (1669/GO/abc/12/CPCSEA). One hundred sixty-eight-day old healthy broiler chicks (vencobb) were procured from a local market. These chicks were weighed and divided into two dietary treatment groups (A and B) containing eighty-four birds each. Both the groups of birds were raised on the deep litter from day one to 35th day of age. Throughout the experimental period twenty-four-hour light was provided. The chicks were reared in the departmental animal house under strict hygienic conditions and were given ad libitum feed and water. The feeding was done in three phases i.e. pre starter (0-14 days), starter (15-28 days) and finisher (29-35 days) phase. The feed was procured from market i.e. Venkys' pelleted feed with specifications mentioned in the Table 1.

Table 1: Venky's pelleted feed composition and specifications

Age in Days	0-14 Days	15 – 28 Days	29 – 35 Days
Feed Type	Pre-Starter	Starter	Finisher
Energy, Kcal/KG	3000	3125	3250
Crude Protein%	22.5	21	19.5
Crude Fibre%	<4	<4	<4
Calcium%	0.94	0.92	0.88
Av. Phosphorous%	0.45	0.42	0.4
Dig Lysine%	1.25	1.1	1
Dig Methionine%	0.57	0.53	0.49

Dry fruits of amla were collected from local market and it was grinded and made into powder form. It was mixed equally as a feed supplement in the procured broiler ration throughout all the phases' i.e. pre-starter, starter and finisher at the rate of 10g/kg of feed in the diet of group A broiler birds; whereas group B broiler birds were given feed and water devoid of dry fruit extract of amla supplementation throughout the experiment. The water provided to all the chicks was boiled and subsequently cooled. After rearing for one week chicks of both the groups (A and B) were again divided into two subgroups (group A into A1 & A2 and group B into B1 & B2) containing 48 and 36 birds each, respectively. At the age of 7 days' chicks of group A1 and B1 were injected intraperitoneal with standard infective dose of pathogenic *E. coli* (i.e. at the rate of 10^7 CFU of *E. coli*/0.5 ml). During the experiment birds were observed for weekly body weight gain, clinical signs and mortality if any.

E. coli (serotype O120) isolated from naturally infected cases, was inoculated into Brain Heart Infusion (BHI) broth and incubated at 37°C for 24 h. Viable count of *E. coli* organism per ml of BHI was determined

by surface spread method as described certain group of scientists (Cruickshank *et al.*, 1975). Serial 10 fold dilutions of the above culture were prepared in the sterile phosphate buffer saline (PBS) and 0.1ml of each dilution was pipetted onto three MacConkey's Lactose Agar (MLA) plates. The inoculum on the plates was spread with the help of a sterile spreader and then these plates were incubated at 37°C for 24 h. The average count of three plates of particular dilution having colonies in the range of 30-300 was calculated. This bacterial count for particular dilution was made in 0.1 ml, the inoculum that was used for each dilution. Then the viable count per ml was determined which was considered as Colony Forming Units (CFU) of the *E. coli*. The infective dose at the rate of 10⁷ CFU of *E. coli*/0.5 ml was prepared that was used for the experiment as *E. coli* inoculum (Kumar *et al.*, 2003).

Per cent Protective Effect Due to Mortality

Overall per cent mortality in infected groups (B1) –

$$\frac{\text{Overall per cent mortality in amla supplemented group (A1)}}{\text{Overall per cent mortality in infected group (B1)}}$$

The data for various parameters were subjected to statistical analysis by using Duncan Multiple Range Test as modified by Kramer (1957) at 5 per cent level of significance using SPSS 20.0 version software. Individual means were compared for statistical significance using least significance difference. However, the statistical significance, where the comparison was made between two means, was assessed by independent samples "T" test.

Results and Discussion

Globally colibacillosis is encountered as a common enteric disease that carries a significant economic importance concerning the loss of poultry flocks. It is the most common infectious bacterial disease of poultry. Colibacillosis is caused by infection with the bacteria *Escherichia coli*. It is associated with many different kinds of diseases ranging from respiratory tract infection to swollen head syndrome in poultry to urinary tract infections. *E. coli* can infect hosts as either a primary or secondary pathogen. The present experiment was undertaken in broiler chicks to evaluate the effect of dry fruit extract of amla supplementation on colibacillosis with respect to its clinical signs, mortality and body weight gain. No clinical signs were observed in both the control groups A2 and B2 throughout the experiment. Clinical signs of *E. coli* infection in the group B1 started to appear at 12 hours post infection. These clinical signs were dullness, depression, drooping of the head and neck, closing of eyes and ruffled feathers (Fig. 1). They were huddling together near the heat source (Fig. 2). Thereafter the chicks showed anorexia, listlessness, inappetance, ruffled feathers and closing of eyes. The birds showed outstretching and drooping of wings and they were not able to bear weight on their legs (Fig. 3) and some of the infected birds exhibited

respiratory distress and diarrhoea which was watery, pasty white and bloody (Fig. 4) in few birds soiling the vent and resulting in dehydration.



Figure 1: Group B1: 3 DPI: Bird showing depression, closing of eyes and ruffled feather



Figure 2: Group B1: 3DPI: huddling near heat source



Figure 3: Group B1: 5 DPI: unable to bear weight, flattened wings



Figure 4: Group B1: 5 DPI: white pasty bloody diarrhea

These clinical signs were more severe at 5 DPI. Thereafter the severity of clinical signs in survived birds was started to decline and they were significantly reduced from 18 DPI. There was almost complete recovery in clinical signs at 21 DPI. The clinical signs observed in the present experimental studies like dullness, depression, weakness, inappetance, ruffled feathers, closing of eyes, drooping of head and neck, respiratory distress, watery and white pasty diarrhoea, dehydration and huddling near the heat source were in accordance with the earlier studies conducted by different other workers (Srinivasan *et al.*, 2003; Saini,

2004; Ask *et al.*, 2006; Barnes *et al.*, 2008; Shaheen and El-Far, 2013) in natural and experimental cases of colibacillosis.

On the other hand clinical signs of *E. coli* infection in group A1 in which starter ration was supplemented with 10g/kg dry fruit extract of Amla started appearing at 24 hours post infection. Clinical signs were almost identical to those observed in group B1 but the severity of the signs was of less intensity as compared to group B1 (Fig. 5, 6). The survived birds in group A1 appeared almost normal after 14 DPI. Therefore, the clinical signs of colibacillosis in dry fruit extract of amla supplemented group were of mild degree at different intervals, appeared later and persisted only for shorter period as compared to non-supplemented group. These results indicate that amla has protective effect on disease manifestation which might be due to its immunomodulatory effect (Bharath *et al.*, 2011). Eevuri and Putturu (2013) reported that extracts of Aloe spp, *Lilokha*, *Mondia whytei*, turmeric, tulsi, amla and *Azadirachta indica* exhibited significant antimicrobial activities against *Salmonella typhi*, *Staphylococcus aureus* and *Escherichia Coli*.



Figure 5: Group A1: 3 DPI: Chicks Showing huddling near heat source



Figure 6: Group A1: 3 DPI: closing of eyes

The mortality pattern in different experimental groups is graphically represented in Fig. 7. Mortality in group B1 started from 24 hours post infection and there was death of twelve birds. Thereafter eight birds died on 2 DPI, two on 3DPI, one each on 4, 5, 6, 7, 8 and 10 DPI. Total numbers of birds died in group B1 throughout the experiment were 28 and overall mortality was 58.33 per cent. This result is in accordance to the study of Kemmett *et al.* (2013) who suggested that Avian Pathogenic *E.coli* (APEC) is responsible for the heavy mortality and morbidity in poultry industry. Heavy mortalities due to colibacillosis in poultry occurs during the first week. This observation was well supported by study conducted by Yassin *et al.* (2009) and Olsen *et al.* (2012) who suggested that first week mortalities can account for up to 50 per cent of total flock loses. On the other hand, mortality in group A1 started from 2 DPI and number of birds died at different intervals was considerably less as compared to group B1. Total death in group A1 was 21 and

overall mortality was 43.75 per cent. The difference in mortality between both the infected groups was about 14.58 per cent.

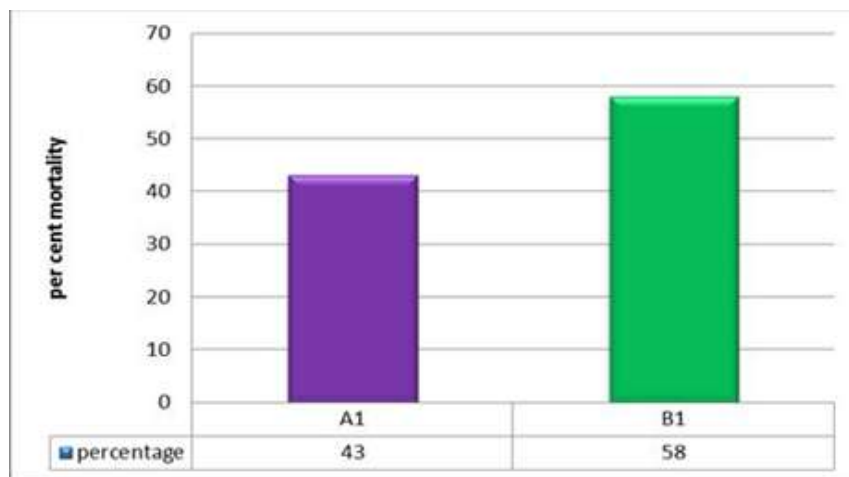


Fig. 7: Mortality of broiler chicks in different experimental groups

These results indicate that the economic losses due to colibacillosis may be reduced to some extent by supplementation of amla in feed. These results are in accordance with the findings of Raheja (2004) and Saini (2004) who reported that neem leaf extract supplementation caused a significant decrease in mortality in broiler chicks infected with *Salmonella gallinarum* and *E. coli*, respectively. Eevuri and Putturu (2013) found that turmeric, tulsi, amla and aloe vera preparations decreased the mortality rates by 13.5 per cent. These results are in accordance with the findings of Kumari (2012) and Sharma (2015) who also reported that tulsi leaf supplementation and neem leaf supplementation respectively caused a significant decrease in mortality in broiler chicks infected with *E. coli* infection. There was no mortality in group A2 and B2 which were non-infected groups. The mortality score or the protective effect due to amla supplementation is graphically represented in Fig. 8. The mean body weights of different groups are illustrated in Fig. 9. The mean body weight values in groups A1, B1, A2 and B2 ranges from 280.83±11.27 to 1566.00±16.10, 251.33±20.33 to 1512.50±17.79, 286.33±10.00 to 2305.16±13.23 and 283.66±23.32 to 2246.83±19.95 respectively. A significant decrease ($P \leq 0.05$) in mean body weight was observed in both the infected groups A1 and B1 as compared to non-infected groups A2 and B2 from 3 days post infection (DPI) onwards. More or less similar results have been reported by other workers due to *E. coli* infection in poultry (Sharma, 1991; Baliarsingh *et al.*, 1993; Saini, 2004). One of the possible reasons might be anorexia observed in the *E. coli* infected groups of the present study. However, this decrease was lesser in group A1 in comparison to group B1 and is significant at 7 DPI.

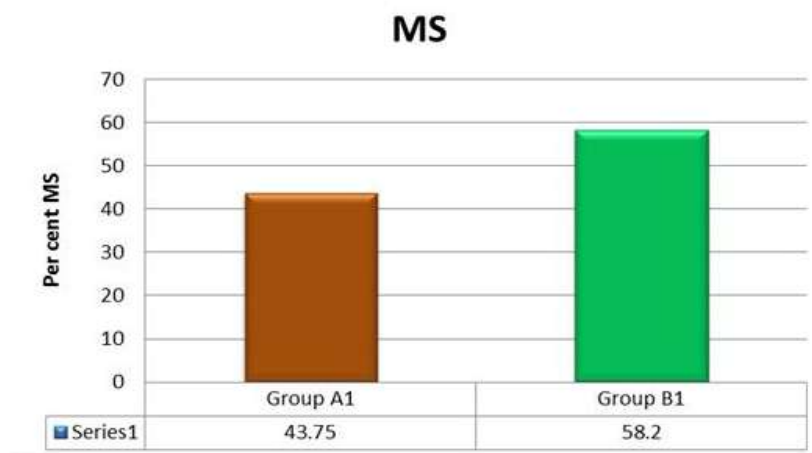


Fig. 8: Overall percent mean mortality scores irrespective of post infection period and organs in different experimental groups

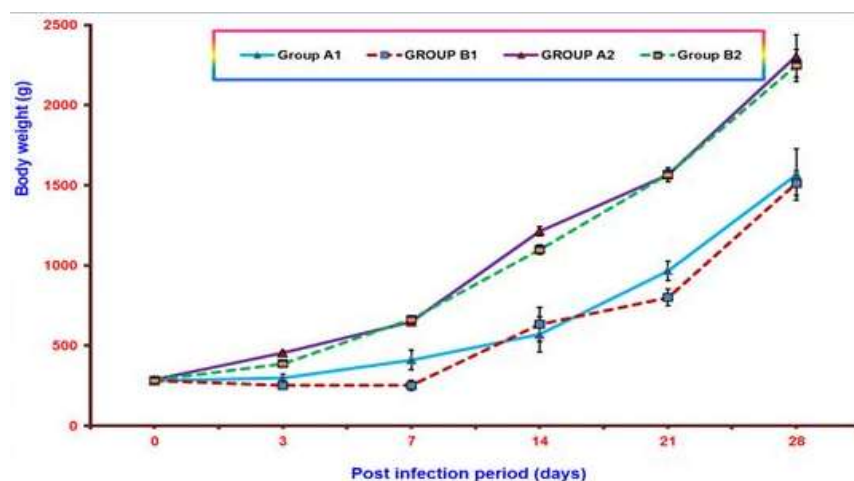


Fig. 9: Mean body weight (g) of broiler chicks in different experimental groups at different intervals

Between the non-infected groups, the mean body weight values were almost similar. Regarding the effect of dry fruit extract of amla on body weight gain, it was noticed that body weight gain in amla supplemented groups (infected as well as non-infected) was significantly higher in comparison to their respective non-aml a supplemented control groups. These results clearly indicate that feeding of amla extract in broiler chickens accelerated growth response of the birds. Mechanism, by which amla supplementation might have stimulated growth, has to be established from different aspects such as growth hormones, enzymes or other factors responsible for cell division or may be the hepatoprotecting effect of amla resulted into improvement of liver function. Enhancement of body weight gain due to dry fruit extracts of amla supplementation has also been reported by other workers in the poultry (Mode *et al.*, 2009; Sanjyal and Sapkota, 2011; Patil *et al.*, 2012). Kumar *et al.* (2013) observed that supplementation of Indian gooseberry (1 per cent), multi-enzyme (0.05 per cent) and their combination (1+ 0.05 per cent) in broiler diet did not affect the body



weight and weight gain of broiler at six weeks of age. Patel *et al.* (2016) observed that the average body weights at the end of the 6th week were significantly higher ($p < 0.05$) in groups supplemented with 0.8 per cent and 0.4 per cent of *E. officinalis* fruit powder as compared to control group. Khetmalis *et al.* (2018) found a significant improvement in body weights of broiler birds supplemented with amla and aflatoxin mixed feed as compared to aflatoxin intoxicated feed.

Conclusion

On the basis of results of the present study it is concluded that supplementation of 10g/kg dry fruit extract of amla in starter ration of broiler feed significantly reduced the severity and recovery period of colibacillosis in chicks as evidenced by clinical signs, mortality and increase in body weight after depletion due to detrimental effect of colibacillosis.

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References

1. Annual report, 2016-17. Department of animal husbandry, dairying & fisheries, Ministry of agriculture, Government of India. New Delhi. Available at: <http://dahd.nic.in/sites/default/files/Annual%20Report%202016-17>.
2. Ask, B., Van Der Waaij, E. H., Van Eck, J. H. H., Van Arendonk, J. A. M. and Stegeman, J. A. (2006). Defining susceptibility of broiler chicks to colibacillosis. *Avian Pathology*, 35(02): 147-153.
3. Baliarsingh, S.K., Rao, A.G. and Mishra, P.R. (1993). Pathology of experimental colibacillosis in chicks. *Indian Veterinary Journal*, 70: 808-812.
4. Barnes, H. J. and Guy, J. S. (2003). Poultry enteritis-mortality syndrome. *Diseases of Poultry*, 11: 1171-1180.
5. Barnes, H. J., Lisa, K. N. and Vaillancourt, J. P. (2008). Diseases of Poultry. 12th ed. Y. M. Saif, H. J. Barnes, A. M. Fadly, J. R. Glisson, L. R. Mc Dougald, L. K. Nolan, and D. E. Swayne, ed. Iowa State University Press, Ames.
6. Bharath, B. K., Anjaneyulu, Y. and Srilatha, C. (2011). Immunomodulatory effect of *Ocimum sanctum* against endosulfan induced immunotoxicity in Wistar Rat. *Veterinary World*, 4(1): 25.
7. Cruickshank, R., Duguid, J. P., Marsion, B. P. and Swain, R. H. A. (1975). Medical-Microbiology Vol II 12th ed., Churchill Livingstone, Edinburgh, London and New York.
8. Eevuri, T. R. and Putturu, R. (2013). Use of certain herbal preparations in broiler feeds-A review. *Veterinary World*, 6(3).
9. Goswami, P., Chakraborty, A., Das, R., Sarkar, P. and Som, T. L. (2004). Isolation and identification of *Escherichia coli* from poultry from field cases. *Indian Veterinary Journal*, 81(8): 951-952.
10. International poultry and livestock expo. 2015. Available at; <http://www.iplexpo.com/>.
11. Kemmett, K., Williams, N. J., Chaloner, G., Humphrey, S., Wigley, P. and Humphrey, T. (2014). The contribution of systemic *Escherichia coli* infection to the early mortalities of commercial broiler chickens. *Avian Pathology*, 43(1): 37-42.



12. Khetmalis, R., More, B., Mote, C., Jadhav, S., Kamdi, B., & Dhaygude, V. (2018). Experimental Study on Pathology of Aflatoxicosis in Broiler Chicks and Its Amelioration by *Emblica officinalis* (Amla) Supplementation. *International Journal of Livestock Research*, 8(10): 287-297.
13. Kramer, C. Y. (1957). Extension of multiple range tests to group correlated adjusted means. *Biometrics*, 13(1): 13-18.
14. Kumar, A., Jindal, N., Shukla, C. L., Pal, Y., Ledoux, D. R. and Rottinghaus, G. E. (2003). Effect of ochratoxin A on *Escherichia coli*-challenged broiler chicks. *Avian Diseases*, 47(2): 415-424.
15. Kumar, M., Sharma, R. K., Chaudhari, M. and Jakhar, A. (2013). Effect of Indian gooseberry and multi-enzyme supplementation on the performance of broilers during hot weather. *Haryana Vet*, 52: 66-68.
16. Kumari, M (2012). Clinico-pathological and Immunological studies in *Escherichia coli* infected broiler chicks fed on *Ocimum sanctum* leaf supplemented feed. M.V.Sc Thesis, LLRUVAS, Hisar.
17. Lutful Kabir, S. M. (2010). Avian colibacillosis and salmonellosis: A closer look at 403 epidemiology, pathogenesis, diagnosis, control and public health concerns. *International Journal of Environmental Research and Public Health*, 7: 89-114.
18. Mettifogo, E., Nuñez, L. F., Chacón, J. L., Santander Parra, S. H., Astolfi-Ferreira, C. S., Jerez, J. A., and Piantino Ferreira, A. J. (2014). Emergence of enteric viruses in production chickens is a concern for avian health. *The Scientific World Journal*, <https://doi.org/10.1155/2014/450423>.
19. Mode, S. G., Funde, S. T., Waghmare, S. P. and Kolte, A. Y. (2009). Effect of herbal immunodulator on body weight gain in immunosuppressed broiler birds. *Veterinary World*, 2(7): 269 – 270.
20. Olsen, R. H., Frantzen, C., Christensen, H. and Bisgaard, M. (2012). An investigation on first-week mortality in layers. *Avian Diseases*, 56(1): 51-57.
21. Patel, A. P., Bhagwat, S. R., Pawar, M. M., Prajapati, K. B., Chauhan, H. D. and Makwana, R. B. (2016). Evaluation of *Emblica officinalis* fruit powder as a growth promoter in commercial broiler chickens. *Veterinary World*, 9(2): 207.
22. Patil, S. G., Deshmukh, A. A., Padol, A. R. and Kale, D. B. (2012). In vitro antibacterial activity of *Emblica officinalis* fruit extract by tube dilution method. *International Journal Toxicology Applied Pharmacology*, 2(4): 49-51.
23. Raheja, S. (2004). Studies on the effect of neem (*Azadirachta indica*) leaf extract on the pathology of experimental fowl typhoid in broiler chickens. M.V.Sc Thesis, CCSHAU, Hisar.
24. Saini, V. (2004). Studies on the effect of neem (*Azadirachta indica*) leaf extract on the pathology and pathogenesis of *E. coli* infection in broiler chicks. M.V.Sc. thesis, CCS Haryana Agricultural University, Hisar.
25. Sanjyal, S. and Sapkota, S. (2011). Supplementation of broilers diet with different sources of growth promoters. *Nepal Journal of Science and Technology*, 12: 41-50.
26. Shaheen, H. M. and El-Far, A. H. (2013). Evaluation of the therapeutic efficacy of pefloxacin and florfenicol combination in broilers experimentally challenged by *Escherichia coli*. *International Journal of Pharmacology Science Review and Research*, 23: 396-404.
27. Sharma, D. (1991). Studies on experimental colibacillosis in broiler chicks with *Escherichia coli* serotype O2. M.V.Sc Thesis, CCS Haryana Agricultural University, Hisar.
28. Sharma, V. (2015). Pathological and immunological studies of *Escherichia coli* infection in broiler chicken with special reference to effect of neem (*Azadirachta indica*) leaf extract. M.V.Sc Thesis, LLRUVAS, Hisar.
29. Singh, S., Atkare, S. S., Bhardwaj, J. K., Baghel, R. P. S. and Jain, A. K. (2010). Performance of broiler reared in different stocking density and fed protein as well as amla (*Emblica officinalis*) supplemented ration. *Indian Journal of Animal Production and Management*, 26(1/2): 53-55.
30. Srinivasan, P., Rao, G. S. and George, V. T. (2003). Serotyping of *Escherichia coli* isolated from natural cases of colibacillosis in chicken in and around Namakkal. *Indian Veterinary Journal (India)*.
31. Suresh, S., Morton, M. and Prabhu M. (1990). Disease patterns and incidence in 1989- An outlook. *Poultry Guide*, 27(5): 83.



32. Vandemaele, F., Vereecken, M., Derijcke, J. and Goddeeris, B.M. (2002). Incidence and antibiotic resistance of pathogenic *Escherichia coli* among poultry in Belgium. *Veterinary Record*, 151: 355-356.
33. Y.M. Saif. (2008). "Viral enteric infections," in *Diseases of Poultry*, Y. M. Saif, A. M. Fadly, J. R. Glisson, L. R. McDougald, L. K. Nolan, and D. E. Swayne, Eds., pp. 329–330, Blackwell, Ames, Iowa, USA.
34. Yassin, H., Velthuis, A. G., Boerjan, M. and van Riel, J. (2009). Field study on broilers' first-week mortality. *Poultry Science*, 88(4): 798-804.

