

*Clinical Article*

**POLYHERBAL FORMULATION AND ITS ROLE IN ALLEVIATING  
SIGNS OF DIARRHOEA IN BROILER BIRDS**

**Mhase Prashant Patilba<sup>1</sup>, Anurag Borthakur<sup>2</sup>, Vikas Yadav<sup>2</sup>, K. Ravikanth<sup>2</sup>  
and Shivi Maini<sup>2</sup>**

<sup>1</sup>Assistant Professor, Dept. of Veterinary Microbiology, Krantisinha Nana Patil College of  
Veterinary Science, Shirwal, Maharashtra-412801

<sup>2</sup>Research and Development (R&D), Ayurved Limited, Baddi, Himachal Pradesh  
E-mail: clinical@ayurved.in (\*Corresponding Author)

**Abstract:** A total of 120 broiler birds were taken for the purpose of study and allotted into four different groups having 30 birds in each. Group T0 was kept as negative control. Group T1 was kept as positive control and challenged with *E.coli* on 8<sup>th</sup> day and maintained on non medicated feed. Group T2 was treated with Salcochek at the rate of 500 g/tonne of feed (Prophylactic dose) with concomitant challenge with *E.coli* on 8<sup>th</sup> day. Group T3 was treated with Salcochek at the rate of 1000g/tonne of feed (Therapeutic dose) with concomitant challenge with *E.coli* on 8<sup>th</sup> day. Parameters viz. performance traits, clinical symptoms, mortality, TVC of *E.coli* in faecal samples, haemagglutination inhibition titre, gross and histopathological changes were evaluated. Results revealed that there was significant improvement in the mean weekly body weight and mean weight gain in the Salcochek (M/S Ayurved) treated groups T2 and T3 as compared to the positive control group T0. Mortality % was significantly less in the Salcochek treated groups as compared to control group. Total viable *E.coli* count in faeces was also significantly reduced in the Salcochek treated groups as compared to control group. The Hemagglutination inhibition titre was significantly higher in the Salcochek treated groups as compared to control group. Thus, it can be inferred that Salcochek is highly effective in alleviating symptoms of diarrhea and promote growth in birds.

**Keywords:** Body weight, Hemagglutination Inhibiton Titre, TVC, Diarrhoea.

## INTRODUCTION

Enteric diseases are one of the most important problems in the poultry industry because of high economic losses due to decreased weight gain, increased mortality rates, worse feed conversion ratio, greater medication costs, and increased risk of contamination of poultry products for human consumption (Timbermont *et al.*, 2011). Colibacillosis is one of the most important diseases threatening the poultry industry. (Ibrahim 1998, Ewers 2005, Ayoub 2007). Antimicrobial agents are used extremely in order to reduce the great losses caused by *Escherichia coli* infections in poultry industry (Talebiyan *et al.*, 2014). However, there are

a number of publications that suggest a link to the use of antibiotics in animal production to an increase in human infections with antibiotic-resistant bacteria (Simonsen *et al.*, 1998; Klare *et al.*, 1999; and Bogaard *et al.*, 2001). The alarming challenge facing physicians and pharmacist now, is the need to develop alternative approaches in addition to the search for new antimicrobial compounds (Sibanda and Okoh, 2007). Thus, both conventional and organic poultry production need alternative methods to improve growth and performance of poultry. Plants might hold a promise for combating the problem of antibiotic resistance. Herbs, spices, and various other plant extracts are being evaluated as alternatives to antibiotics and some do have growth promoting effects, antimicrobial properties, and other health-related benefits (Diaz-Sanchez *et al.*, 2015). Several herbal plants viz. *Aegle marmelos*, *Holarrhena antidysentrica* have been known to possess anti- diarrheal property and are serious candidates as alternatives to antibiotic therapy (Brigesh *et al.*, 2009, Rao 2012, Kavitha *et al.*, 2004, Sharma *et al.*, 2007). Thus, the present study has been undertaken to evaluate the effect of herbal anti- diarrheal in the treatment of *E. coli* induced diarrhoea in poultry.

## MATERIALS AND METHODS

### Experimental design

The present study was carried out in the Department of Microbiology, Krantisinh Nana Patil College of Veterinary Science, Shirwal during the period of 2016-17 to evaluate the prophylactic and therapeutic efficacy of herbal anti- diarrhoeal Salcochek (M/S Ayurved Limited) against diarrhoea in broilers. A total of 120 healthy vaccinated commercial broiler chicks weighing between 45-55 g were procured from Ms/- Venkateswara hatchery. The broilers were allotted into four different groups having 30 birds in each. Group T0 (n=30) was kept as negative control and fed standard basal diet. Group T1 (n=30) was kept as positive control and challenged with 0.4 ml *E. coli* inoculum (approx. bacterial count  $1 \times 10^4$  CFU/ml) on 8<sup>th</sup> day of age and maintained on a non- medicated diet. Group T2 was supplemented with Salcochek at a prophylactic dose of 500g/tonne of feed from 0 day to 35<sup>th</sup> day along with concomitant challenge with 0.4 ml *E. coli* inoculum (approx. bacterial count  $1 \times 10^4$  CFU/ml) on 8<sup>th</sup> day. Group T3 was supplemented with Salcochek at a therapeutic dose of 1000g/tonne of feed from 0 day to 35<sup>th</sup> day along with concomitant challenge with 0.4 ml *E. coli* inoculum (approx. bacterial count  $1 \times 10^4$  CFU/ml) on 8<sup>th</sup> day. Performance parameters viz. weekly body weight, body weight gain were recorded. Microbiological studies viz. total viable *E.coli* count, Haemagglutinin inhibition titre were also carried out. Gross and

histopathological examinations of various organs were conducted at the end of the experiment *i.e.* on 33<sup>rd</sup> day.

### Statistical analysis

All the results were analyzed statistically by analysis of variance to determine the means and standard error as per the methods described by Snedecor and Cochran (Snedecor, 1994)

## RESULTS AND DISCUSSION

### Weekly body weight

Results revealed that the mean weekly body weight was significantly higher in the Salcochek (therapeutic dose) treated group T3 (1916 g) and Salcochek (prophylactic) treated group T2 (1914 g) as compared to positive control group T1 (1311 g) at the end of 5<sup>th</sup> week (table 1). The weekly body weight in the 5<sup>th</sup> week was non- significantly variable between the Salcochek treated groups T3 and T2 as compared to negative control (non- infected) group T0. This indicates remarkable effect of Salcochek in enhancing body weight in broilers infected with *E.coli*. The increase in weekly body weight may be attributed to the presence of *Aegle marmelos*, a constituent ingredient of Salcochek which is known to have anti-diarrheal effect (Gricilda, 2001), and also stomachic and digestive properties (Tripathi, 2011).

**Table 1. Weekly body weight (g) of broilers in control and treated groups**

Age of birds	Group T0	Group T1	Group T2	Group T3
1 <sup>st</sup> Week	184±0.068	177±0.076	175±0.074	170±0.174
2 <sup>nd</sup> Week	458±0.065	299±0.16	443±0.06	442±0.116
3 <sup>rd</sup> Week	896±0.136	521±0.19	844±0.0412	846±0.102
4 <sup>th</sup> Week	1412±0.114	867±0.18	1341±0.071	1337±0.089
5 <sup>th</sup> Week	2002±0.159 <sup>a</sup>	1311±0.16 <sup>b</sup>	1914±0.0649 <sup>a</sup>	1916±0.115 <sup>a</sup>

### Weekly body weight gain

The weekly body weight gain was significantly higher in the Salcochek (therapeutic dose) treated group T3 (87 g) and Salcochek (prophylactic dose) treated group T2 (85 g) as compared to positive control (infected) group T1 (68 g) at the end of 5<sup>th</sup> week (table 2). The body weight gain was non- significantly variable between the control (non- infected) group T0 and Salcochek treated groups T2 and T3 at the end of 5<sup>th</sup> week. This finding is in

consonance with observations of Abdelsamie (1983), Oke and Oke (2007), who also reported an increase in weight gain following the administration of *Aegle Marmelos* extract.

**Table 2. Weekly body weight gain (g) of broilers in control and treated groups**

Age of birds	Group T0	Group T1	Group T2	Group T3
1 <sup>st</sup> Week	27±0.11	28±0.04	29±0.15	28±0.22
2 <sup>nd</sup> Week	50±0.20	18±0.03	47±0.14	46±0.10
3 <sup>rd</sup> Week	67±0.12	39±0.04	66±0.17	63±0.12
4 <sup>th</sup> Week	78±0.12	58±0.02	77±0.16	76±0.11
5 <sup>th</sup> Week	88±0.10 <sup>a</sup>	68±0.09 <sup>b</sup>	85±0.10 <sup>a</sup>	87±0.09 <sup>a</sup>

### Mortality

The mortality percentage was significantly reduced in the Salcochek (therapeutic) treated group T3 (1.12 %) and Salcochek (prophylactic dose) treated group T2 (1.67%) as compared to the positive control (infected) group T1 (3.89%) (table 4). The decreased mortality may be attributed to the immunomodulatory effect of *Plantago ovata*, a constituent ingredient of Salcochek, which is rich in flavonoids and polyphenols (Haddadian, 2014). The astringent property conferred by *Holarrhena antidysentrica*, a constituent ingredient of Salcochek, helps to ameliorate the deleterious effects of dehydration thereby reducing the mortality rate (Zope *et al.*, 2016).

**Table 3. Mortality % of broilers in the control and treated groups.**

Groups	T0	T1	T2	T3
Mortality %	0.56	3.89	1.67	1.12

### Total viable E.coli count (mean log<sub>10</sub> cfu/ml)

The mean cfu on completion of trial after 28<sup>th</sup> day after completion of trial in the Salcochek (therapeutic dose) treated group T3 ( $227 \times 10^4$ ) and Salcochek (prophylactic dose) treated group T2 ( $186 \times 10^4$ ) was significantly reduced as compared to the positive control (infected) group T1 ( $251 \times 10^6$ ). Log<sub>10</sub>cfu/ml on completion of trial after 28<sup>th</sup> day in the Salcochek (therapeutic dose) treated group T3 (-648) and Salcochek (prophylactic dose) treated group T2 (-736) was significantly less as compared to positive control (infected) group T1 (1.394) (table 4). The decrease in the mean cfu and log<sub>10</sub>cfu/ml in the Salcochek treated groups may

be due to the anti bacterial property of *Aegle marmelos*, a constituent ingredient of Salcochek (Poonkothai, 2008).

**Table 4. Results of total viable *E. coli* count (mean log<sub>10</sub> cfu/ml) in the control and Salcochek treated groups**

On 3 <sup>rd</sup> day after challenge				
	T0	T1	T2	T3
Mean cfu	238 x 10 <sup>3</sup>	247 x 10 <sup>5</sup>	280 x 10 <sup>6</sup>	136 x 10 <sup>6</sup>
Log <sub>10</sub> cfu/ml	-1.62	1.382	1.44	1.36
On completion of trial after 28 <sup>th</sup> day.				
Mean cfu	256 x 10 <sup>3</sup>	251 x 10 <sup>6</sup>	186 x 10 <sup>4</sup>	227 x 10 <sup>4</sup>
Log <sub>10</sub> cfu/ml	-1.589	1.394	-0.736	-0.648

### Haemagglutination inhibition titre

The mean titre on 28<sup>th</sup> day was significantly higher in the Salcochek (therapeutic dose) treated group T3 (1:41.6) and Salcochek (prophylactic dose) treated group T2 (1:38.4) as compared to positive control (infected) group T1 (1:12). The mean log<sub>2</sub>/ml on 28<sup>th</sup> day in the Salcochek (therapeutic dose) treated group T3 (5.3±0.132) and Salcochek (prophylactic dose) treated group T2 (5.2± 0.132) was significantly higher as compared to positive control (infected) group T1(3.5±0.17) (table 5). This may be ascribed to the immunomodulatory effect of *Acacia catechu*, present in Salcochek (Ismail, 2009).

**Table 5: Results of Haemagglutination inhibition titre (mean log<sub>2</sub> antibodies/ml) of control and treated groups**

Before Vaccination				
Groups	Group T0	Group T1	Group T2	Group T3
Mean titre	1:14.4	1:12	1:13.6	1:12.8
Mean log <sub>2</sub> /ml	3.8±0.133	3.5±0.167	3.7±0.153	3.6±0.165
After vaccination on 28 <sup>th</sup> day				
Mean titre	1:48	1:12	1:38.4	1:41.6
Mean log <sub>2</sub> /ml	5.5+0.164 <sup>b</sup>	3.5 + 0.17 <sup>c</sup>	5.2 + 0.132 <sup>b</sup>	5.3 + 0.132 <sup>b</sup>

### CONCLUSION

The body weight was significantly increased in the Salcochek treated groups as compared to positive control due to its ameliorative effect on diarrhoea. The total viable *E. coli* count was

also significantly less in faecal samples of Salcochek treated groups as compared to positive control. The results are highly suggestive of profound anti diarrheal effect of Salcochek on *E.coli* induced diarrhoea.

#### ACKNOWLEDGEMENT

The authors are thankful to Ayurved Limited, Baddi, India and Dept. of Department of Microbiology, Krantisinha Nana Patil College of Veterinary Science, Shirwal for providing the necessary facilities to carry out the research work.

#### REFERENCES

- [1] Abdelsamie, R.E., Ranaweera, K.N., Nano, W.E. 1983. The influence of fibre content and physical texture of the diet on the performance of broilers in the tropics. *British poultry Science*. 24 (3): 383-90
- [2] Ayoub, M.A.M. 2007: Studies on epidemiology of *E.coli* in some poultry farms. M.V.Sc thesis. Faculty of vet. Med, Alexandria University.
- [3] Bogaard, A., van den, E., London, N., Driessen, C. and Stobberingh, E. E. 2001. Antibiotic resistance of faecal *Escherichia coli* in poultry, poultry farmers, and poultry slaughterers. *Journal of Antimicrobial Chemotherapy*. 47:763–771.
- [4] Brijesh, S., Daswani, P., Tetali, P., Antia, N. and Birdi, T. 2009. Studies on the antidiarrhoeal activity of *Aegle marmelos* unripe fruit: Validating its traditional usage. *Complementary and Alternative Medicine*.
- [5] Debnath, B.C., Ravikanth, K., Thakur, A. and Maini, S. 2014. Herbal antidiarrhoeal formulation for the treatment of weaning diarrhoea in piglets. *World journal of pharmacy and pharmaceutical sciences*. 3(6): 21-23.
- [6] Diaz-Sanchez, S., D'Souza, D., Biswas, D. and Hanning, I. 2015. Botanical alternatives to antibiotics for use in organic poultry production. *Poultry Science*. 94(6): 1419-1430.
- [7] Ewers, C., Janssen, T., Kiessling, S., Philip, H.C., and Wieler L.H. 2005. Rapid detection of virulence associated genes in avian pathogenic *E.coli* by multiplex polymerase chain reaction, *Avian Diseases*.49 :269-273
- [8] Gricilda, F. and Thomas, M. 2001. Study of antidiarrhoeal activity of four medicinal plants in castor oil induced diarrhea. *Journal of Ethnopharmacology*. 76: 73-76.
- [9] Haddadian, K., Haddadian, K. Zahmatkash, M. 2014. A review of plantago plant. *Indian Journal of traditional knowledge*. 13:4
- [10] Ibrahim, F.A. 1998. Studies on ground water and possible health risks in south Sinai, Egypt, *SCVMI*, 1(1):141-150

- [11] Ismail, S. and Asad, M. 2009. Immunomodulatory activity of *Acacia catechu*. *Indian Journal of Physiology and Pharmacology*. 53(1): 25-33.
- [12] Kavitha, D., Shilpa, PN, Devaraj SN. 2004. Antibacterial and antidiarrhoeal effects of alkaloids of *Holarrhena antidysenterica*. *Indian Journal of Experimental Biology*. 42 (6): 589-94
- [13] Klare, I., Badstübner, D., Konstabel, C., Böhme, G., Claus, H. and Witte, W. 1999. Decreased incidence of vanA-type vancomycin-resistant enterococci isolated from poultry meat and from fecal samples of humans in the community after discontinuation of avoparcin usage in animal husbandry. *Microbial Drug Resistance*. 5:45–52.
- [14] Oke, D.B. and Oke, M.O. 2007. Effects of feeding graded levels of sawdust obtained from *Daniellia ogea* tree on the performance and carcass characteristics of broiler chickens. *Research Journal of poultry sciences*. 1(1): 12-15.
- [15] Poonkothai, M. and Saravanan M. 2008. Antibacterial activity of *Aegle marmelos* against leaf, bark and fruit extracts. *Anc Life Science*. 27(3): 15-18.
- [16] Rao, H.J.G. and Lakshmi P. 2012 Evaluation of anti-diarrhoeal activity of extract from leaves of *Aegle marmelos*. *Journal of applied pharmaceutical science*. 2(02): 75-78.
- [17] Sharma, D.K., Gupta V.K., Kumar, S., Joshi, V., Shankar, R., Mandal, K., Prakash, A.G.B and Singh, M. 2015. Evaluation of antidiarrhoeal activity of ethanolic extract of *Holarrhena antidysenterica* seeds in rats. *Vet world*. 8(12): 1392-1395.
- [18] Sibanda, T. and Okoh, A. I. 2007. The challenges of overcoming antibiotic resistance: Plant extracts as potential sources of antimicrobial and resistance modifying agents, *African Journal of Biotechnology*. 6(25):2886-2896.
- [19] Simonsen, G. S., Haaheim, H., Dahl, K. H., Kruse, H., Løvseth, A., Olsvik, Ø. and A. Sundsfjord A. 1998. Transmission of vanA-type vancomycin-resistant enterococci and vanA resistance elements between chicken and humans at avoparcin-exposed farms. *Microbial Drug Resistance*. 4:313–318.
- [20] Snedecor, G.W. and Cochran, W.G. 1994. *Statistical methods*. 8<sup>th</sup> ed. IOWA: IOWA State University Press. 1-503.
- [21] Talebiyan, R., Kheradmand, M., Khamesipour, F., Faradonbeh, M.R. 2014. Multiple antimicrobial resistance of *E. coli* isolated from chickens in Iran. *Veterinary Medicine International*. Article ID 491418.
- [22] Timbermont, L., Haesebrouck, F., Ducatelle, R. and Van Immerseel, F. 2011. Necrotic enteritis in broilers: an updated review on the pathogenesis. *Avian Pathology*. 40:341-347

- [23] Tripathi, S. 2011. Total Gut Integrity in Poultry and Synergistic Role of Herbs. *International Animal Health Journal*. 3(1).
- [24] Zope, R.A., Shrikanth, P., Kumar, K.N.S., Ravikrishna, S. and Bairy, T.S. 2016. Comparative pharmacognosy atlas of Pum Kutaja (*Holarrhena antidysenterica* Wall. Ex A. Dc.) And Stree Kutaja (*Wrightia tinctoria* (Roxb.) R. Br.). *Journal of Pharmacognosy and Phytochemistry*, 5(3): 160-168.