Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2021.005

RESEARCH ARTICLE

Screening of the Herbs and Evaluation of their Combined Effects on the Health and Immunity of Coccidiosis Challenged Broiler Chickens

Ahmed Ali Moryani[1], Nasir Rajput[1*], Muhammad Naeem[1], Atta Hussain Shah[1] and Ali Raza Jahejo[1,2]

[1]Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University Tandojam, Sindh 70060, Pakistan
[2]College of Veterinary Medicine, Shanxi Agricultural University, Jinzhong 030801, China
*Corresponding author: drnasirrajput@yahoo.com

ARTICLE HISTORY (20-534)

Received: October 13, 2020
Revised: December 03, 2020
Accepted: December 10, 2020
Published online: January 11, 2021

Key words:
Broiler
Coccidiosis
Herbs
TLRs
Immunity

ABSTRACT

The present study was designed to evaluate the efficiency of herbs on the immunity related-genes and health of broiler chickens challenged with coccidiosis. Total (one-day-old, 450) broiler chickens were allotted into 9 groups (n = 50/group). Group A controlled without supplementation, Group B, C, D, E, F, and G were supplemented with *Aloe barbadensis* (5ml/L), *Tinaspora Cordifolia* (20g/L), *Bambusaarundinacea* (5g/L), *Emblica officinalis* (10g/L), *Ferulafoetida regal* (500mg/L), *Tamarindusindica* (50mg/L) respectively. Group H and I were supplemented with a mixture of all 6 herbs/plants at 2ml/L; mixed with distilled water and another with citric acid. Anticoccidial effect of different herbs on comparative average values of bloody diarrhea score, lesion score, oocysts score, and survival percentage were recorded. The survival rate of coccidiosis challenged broiler chickens were observed significantly ($P<0.05$) high in group B, H, and I. Higher level of intestinal histomorphology, blood hematology, *E. coli*, and Lactobacillus observed ($P<0.05$) in group H and I. Antibody titer results (ND and H7) suggested that the titer was high for group B and I. Hormone profiling results of T3, T4 and AST was observed higher in group H. mRNA expression of toll-like receptors 1,2, 3, 4, 5 (TLR1, TLR2, TLR3, TLR4, TLR5) genes were validated on RT-PCR, which were significantly different ($P<0.05$) upregulated in group H and I. In conclusion, supplementation of herbs mixture (Group H and I) at the dose 2ml/L to the coccidiosis challenged broiler chickens overall improved the health and immunity by regulating the mRNA expression of immunity-related toll-like receptors.

To Cite This Article: Moryani AA, Rajput N, Naeem M, Shah AH, Jahejo AR, 2021. Screening of the herbs and evaluation of their combined effects on the health and immunity of coccidiosis challenged broiler chickens. Pak Vet J, 41(2): 228-234. http://dx.doi.org/10.29261/pakvetj/2021.005

INTRODUCTION

Coccidiosis is a parasitic intestinal disease of poultry birds caused by coccidial protozoa. It is a contagious disease infect through contact with contaminated feces and with ingestion of infected tissue. Coccidiosis can cause concurrent infection with bacterial, fungal, and viral pathogens. Coccidiosis is a disease of hot and humid environments caused by different Eimeria species of the phylum Apicomplexa. The disease is characterized by dysentery, bloody diarrhea, enteritis, poor growth, drooping wings, emaciation, and decreased production (Gerhold, 2015).

Additionally, high morbidity and mortality rates are reported, which are further aggravated by concurrent infection. Coccidiosis is considered a disease of poor management (Ali *et al*., 2014; Abbas *et al*., 2019). Most of the coccidiosis-infected birds not show any symptoms, while immature groups having an impaired immune system may endure severe symptoms and fatality (Velkers *et al*., 2010; Shaw *et al*., 2012). Due to the antimicrobial properties and invigorating impact of the herbal plants and their extracted compounds and essential oils on the digestive systems of the animal, these medicinal herbs/plants contribute significant value in the animal diet (Nasir *et al*., 2013).

Erythrocytes of poultry are nucleated and transcriptionally active, well known for their role in the respiration of gasses (Morera *et al*., 2011), and participate in immune regulatory factors of the immune system. *Candida albicans* have been shown to stimulate erythrocytes to release the soluble mediators, which in turn contribute to phagocytic capability (Passantino *et al*., 2007).

Coccidiosis is one of the most economically significant diseases of poultry around the globe (Hussain *et al*., 2017). Depending upon the prevailing market conditions, the total economic losses (treatment, prophylaxis, and supportive therapy) from coccidiosis were estimated to be US $45,405.00. Different species of Eimeria cause an estimated loss of US$ 800 million per year to the world's commercial poultry producers (Williams, 1998). Medicinal plants increase the productive performance of poultry and health generally by stabilizing the microorganisms of the intestine inhibiting the growth of pathogenic microbes and improving the performance and production of digestive enzymes (Takeli *et al*., 2016; Abbas *et al*., 2019). Medicinal herbs each and every plant or their extracts and essential oils consist of many bioactive chemical compounds which can be used as a growth promoter, diuretic, antiparasitic, appetizer, alkaline phosphates stimulator, antibacterial and antifungal agent (Hajati *et al*., 2014; Khater *et al*., 2020; Salman *et al*., 2020; Zaman *et al*., 2020).

The dietary supplementation effect with natural carotenoids curcumin and lutein on the broiler pigmentation and immunity. In addition, we have also found the immunomodulatory effect of *Ocimum basilicum* seeds supplementation to the heat-stressed broiler chickens (Jahejo *et al*., 2019; Zhang *et al*., 2020) and found that it improved the immunity and intestinal absorption. The most common herbs and plant extracts, which are being used as an alternative source of antimicrobials in poultry are aloevera, giloy, tabasheer, vaghayani, anwara, gadamri, garlic, cumin, black cumin, wild mint, pumpkin, thyme, cinnamon, chestnut, clove, alfalfa, turmeric, sumac, mushroom, grape seed, goldthread, mulberry leaf and honeysuckle (Aroche *et al*., 2018). Although, Toll-like receptors expressed in the erythrocytes of chicken (Paolucci *et al*., 2013) and the role of chicken erythrocytes in coccidiosis broiler chickens has not been well documented (Idris *et al*., 2017).

Keeping the significance of herbs in view of the disease prevention efficiency and immunity, the present study has been designed to determine the efficiency of their usage on the immunity-related genes and health of broiler chickens.

MATERIALS AND METHODS

Preparation of Experiment: Present research work is in the continuity of our previous experiment in which the prevalence of common poultry diseases of broiler chicken and influence of different medicinal herbs on the growth was conducted (Moryani *et al*., 2020). In the preliminary study, a survey was conducted at different vicinities of the Sindh province for recording the prevalence of common diseases in broiler and layer farms. For this purpose, a uniform pre-tested questionnaire was used to interview poultry farmers/dealers of Sindh. The herb used and the dose was selected based on the interview conducted in our previous study (Moryani *et al*., 2020).

Experimental design: Four hundred fifty-one-day-old Cobb broiler chickens were obtained from a commercial hatchery and randomly allotted into 9 groups (*n* = 50/group), where broiler chickens were fed different medicinal herbs to evaluate its effect on the immunity and health of broiler chickens on the *in-vitro* study. Group A controlled and given basal diet, which was formulated on the recommendation of the National Research Council (NRC, 1994), to fulfill the nutrient requirements of broiler chicken. Group B was supplemented with leave juice of *Aloe barbadensis* (5ml/L), group C supplemented with the stem of *Tinaspora Cordifolia* (20g/L), group D supplemented with the siliceous secretion of *Bambusa arundinacea* (5g/L), group E supplemented with the fruit of *Emblica officinalis* (10g/L), group F supplemented with gum resin of *Ferulafoetida regal* (500mg/L), group G supplemented with fruit pulp of *Tamarindusindica* (50mg/L). Whereas, group H and I were given a compound mixture of above six herbs/plants mixed in tape water and citric acid respectively and supplemented at 2ml/L. All the nine (9) groups, including group A, were kept as control (fed on the free antibiotic and anticoccidial diet) and provided feed and water *ad libitum*. The Institutional Committee for Animal use and Care, Sindh Agriculture University Tandojam, approved all the experimental procedures.

Coccidiosis infection: All the nine (9) groups, including group-A which was kept as control (fed on the free antibiotic and anticoccidial diet) at the age of 21d, the birds were orally challenged with *Eimeri*a populated oocysts (10'000 oocysts per broiler) diluted in 0.5 ml of distilled water as performed in our previous study (Rajput *et al*., 2014).

Oocyst excretion: A total of ten (10) broiler birds were selected from each group and inspected for bloody diarrhea on the 4th to 6th day of post-inoculation of *Eimeria* (Coccidiosis) parasite infection. Plain for estimating the bloody diarrhea score method, as reported by Youn *et al.* (1993). The technique reported by Zaman *et al*. (2012) was used to rate the score for caecal lesions. The number of oocysts per g of the fecal sample was calculated using the following formula.

$$\text{Number of oocysts excreted} = \frac{X \times 45 \times 10 \times 1/3}{0.15} = X1000$$

Where: X = average count in 1 chamber, 0.15 = volume under ruled area, 45 = total volume of suspension, 10 = 1/10 dilution factor, 1/3 = correction factor for 1g of feces. However, the survival percent of broiler birds was calculated by using the following formula:

$$\text{Survival (\%)} = \frac{\text{Total no. of birds -Total no. of dead birds}}{\text{Total no. of birds}} \times 100$$

Hematology: Blood samples of two birds from each replicate were taken from each group supplemented with different medicinal plants/herbs and non-supplemented control group, during the experiment and process to find the number erythrocytes, white blood cells, hemoglobin, packed cell volume as previously described by Jahejo *et al*. (2019).

Hormone profiling, Alanine Aminotransferase and Aspartate Aminotransferase: Blood samples of two birds were taken from each herb supplemented and non-supplemented control group. Samples were stored for diagnostic analysis of T3 and T4 Hormone profile. Radioimmunoassay was performed in Genesys machine (laboratories Technologies Inc.). Determination Levels of Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) enzymes, which are markers of oxidative damage sustained in hepatic tissue, were measured in the plasma using the corresponding diagnostic kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's protocols.

Antibody titter: According to the description of the World Animal Health Organization, the antibody titer is tested by the hemagglutination test (HA)/hemagglutination inhibition (HI) to check the immunity of birds.

Intestinal microflora: First of all, associated bacteria culture was performed in Nutrient broth media for the enrichment of the bacteria. *Escherichia coli* was cultured on MacConkey agar in aerobic condition and incubated for 24 h at 37°C on the media plates.

$$\text{Bacterial count (cfu/ml)} = \frac{\text{No. of colonies} \times \text{Dilution factor}}{\text{The volume of cultured plate}}$$

Intestinal histomorphology: The process was followed as previously described (Rajput *et al*., 2013; Jahejo *et al*., 2019). The villus height was measured from the tip of the villus to the base.

RNA extraction, cDNA synthesis, and RT-PCR, qPCR: Total RNA extraction of erythrocytes was conducted using TRIzol reagent per manufacturer's protocol. The concentration of the extracted RNA was measured by spectrophotometer (Eppendorf), and the completeness of RNA was rechecked by agarose gel electrophoresis as well as visualization of the 28s and 18s ribosomal RNA, and the procedure was followed as previously described by Jahejo *et al*. (2020) (Table 1). Quantitative real-time PCR was conducted in the QuantStudio6 (Applied Biosystems, American) system using SYBR Green Premix Ex Taq II (TaKaRa, Dalian, China) (Jahejo *et al*., 2020).

Statistical analysis: Statistical analyses were performed using JMP software (SAS USA), and all data were expressed as means values.

RESULTS

Anticoccidial effect of herbs on coccidiosis infection: Anticoccidial effect of different herbs on comparative average values of bloody diarrhea score, lesion score, oocysts score, and survival percentage were recorded for the broiler chickens challenged with coccidiosis (Table 2). Significantly (P<0.05) highest bloody diarrhea, lesion score, and oocyte score were observed in group A for days 4, 5, and 6, whereas the lowest was observed in H and I group. The results reported that a significantly (P<0.05) better survival rate was tested for groups B, H, and I, whereas comparatively worst survival was observed in group A.

Effect of herbs on oocyst excretion: Effect of different herbs on the oocyst excretion of the broiler chickens challenged with coccidiosis on 6, 7, 8, 9, and 10 of post-inoculation days (Table 3). It has been reported that the group H and I had better results significantly (P<0.05) for days 6, 7, 8, 9, and 10, whereas group A and D were found significantly (P<0.05) more oocytes count respectively, for days 6, 7, 8, 9 and 10.

Hematology: Influence of different herbs on hematology of the broiler chickens challenged with coccidiosis (Table 4). It has been reported that RBCs, hemoglobin, WBCs, PCV were found significantly (P<0.05) higher in group I and H, respectively, and lower in group A and B, respectively.

Antibody titter: Effect of different herbs on antibody titter of the broiler chickens challenged with coccidiosis. The antibody titter results of ND were reported significantly (P<0.05) higher in group I, B and lower in group E. The antibody titters result of Avian influenza virus strain H7 were observed significantly (P<0.05) B and I, and lower in group A. The Avian influenza virus strain H9 results were observed significantly (P<0.05) higher in A, B, and lower in group H and I (Fig. 1).

Intestinal microflora: Influence of different herbs on the intestinal microflora of *E. coli* and *Lactobacillus* of the broiler chickens challenged with coccidiosis. The microflora results of *E. coli* were significantly (P<0.05) higher in group A and lower in group H and I. The microflora results of *Lactobacillus* were significantly (P<0.05) higher in group H, I and lower in group A (Fig.1).

Concentration of T3 and T4 hormones: Influence of different herbs on the concentration of Triiodothyronine and Thyroxin hormones of the broiler chickens challenged with coccidiosis (Fig. 2). It has been reported that the concentration of Triiodothyronine hormone was observed significantly (P<0.05) higher in group B and H, whereas lower in group A. The concentration of Thyroxin hormone was observed significantly (P<0.05) higher in group B and lower in group A.

Concentration of AST and ALT enzymes: The influence of different herbs on the concentration of AST and ALT enzymes of the broiler chickens challenged with coccidiosis was observed (Fig. 2). Results reported that the concentration of AST was significantly (P<0.05) higher I and H, however, the concentration was lower in group A. Results of ALT concentration in group A was significantly (P<0.05) higher and lower in group B.

Table 1: Primer sequences used in qPCR

Genes	Primer (5′→3′)	Gene bank accession number
TLR1	F: GATGATACGAAGGTCAGACT R: CAGACTTAGAGGCTCATACA	NM_001007488
TLR2	F:ACCTGGCCCATAACAGGATA R: ATGGAGCTGATTTGGTTGGA	AB046119
TLR3	F: GCCTAAATATCACGGTACTC R: CACAACAGTGGTAGTGATCA	NM_001011691
TLR4	F: AGTCTGAAATTGCTGAGCTCAAAT R: GCGACGTTAAGCCATGGAAG	NM001030693
TLR5	F: CTGCCAAATCTTCGTGTCTT R: ACAGACGGAGTATGGTCAAA	FJ915552
18s rRNA	F: TTCCGATAACGAACGACAC R: GACATCTAAGGGCATCACAG	FM165414

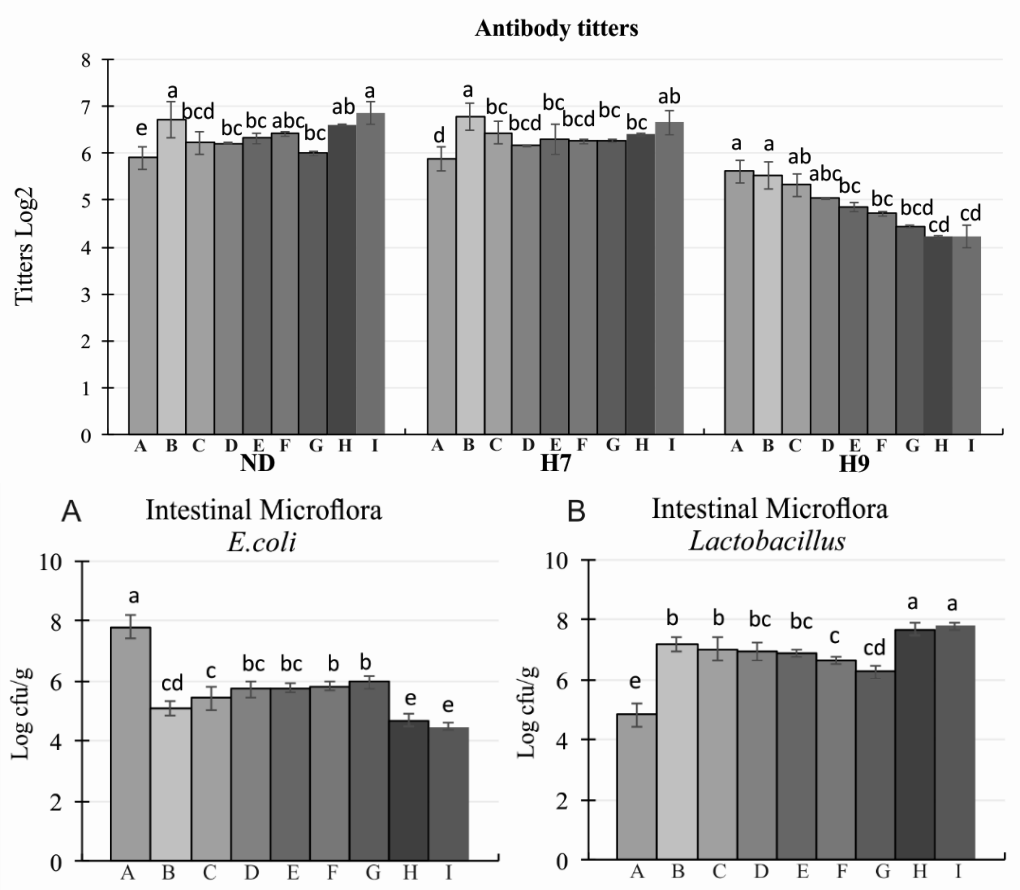

Fig. 1: Influence of herbs on antibody titter of Newcastle disease (ND) and Avian Influenza (AI) and intestinal microflora of broiler chickens challenged with coccidiosis.

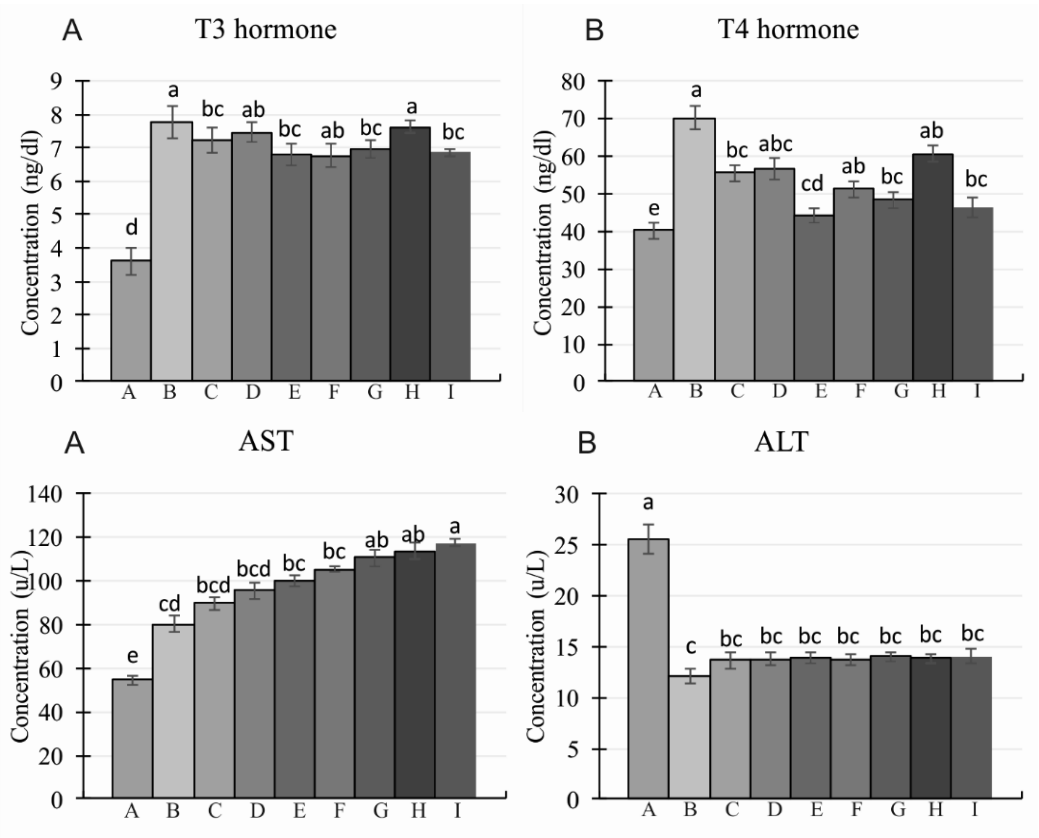

Fig. 2: Influence of herbs on Aspartate aminotransferase (AST) and Plasma alanine aminotransferase (ALT) enzymes, Triiodothyronine and Thyroxin hormones of broiler chickens challenged with coccidiosis: A= Triiodothyronine, B= Thyroxin: A= Aspartate aminotransferase, B= Plasma alanine aminotransferase.

Table 2: Influence of herbs on comparative average values of bloody diarrhea score, lesion score, oocysts score, and survival percentage of broiler chickens challenged with coccidiosis.

Groups	Blood in faces (days post-inoculation)			Lesion score	Oocyst score	Survival rate (%)
	Day 4	Day 5	Day 6			
Group-A	4.8±0.51^{a}	4.1±0.38^{a}	3.5±0.36^{a}	3.7±0.13^{a}	5.2±0.21^{a}	60^{d}
Group-B	0.2±0.32^{d}	0.3±0.12^{d}	0.1±0.32^{d}	0.4±0.12^{c}	0.5±0.33^{d}	100^{a}
Group-C	0.8±0.23^{c}	0.6±0.22^{d}	0.6±0.12^{c}	0.9±0.23^{b}	0.6±0.33^{d}	94^{bc}
Group-D	2.1±0.41^{b}	2.2±0.11^{b}	1.9±0.21^{b}	0.3±0.11^{cd}	1.2±0.11^{b}	100^{a}
Group-E	1.8±0.23^{b}	1.6±0.41^{bc}	1.1±0.15^{bc}	0.4±0.13^{c}	1.0±0.22^{b}	100^{a}
Group-F	1.5±0.22^{b}	1.3±0.11^{bc}	1.2±0.13^{bc}	0.6±0.32^{bc}	0.8±0.22^{c}	99^{a}
Group-G	1.1±0.12^{bc}	1.0±0.12^{c}	0.9±0.33^{c}	0.8±0.12^{b}	0.7±0.32^{c}	95^{b}
Group-H	0^{e}	0^{e}	0^{e}	0^{e}	0^{e}	100^{a}
Group-I	0^{e}	0^{e}	0^{e}	0^{e}	0^{e}	100^{a}

Table 3: Influence of herbs on oocyst excretion of broiler chickens challenged with coccidiosis.

Groups	Oocyst counts (×10^{3}) at different days post-inoculation					
	6	7	8	9	10	Total
Group-A	60±0.21^{a}	97±0.18^{a}	85±0.26^{a}	70±0.18^{a}	51±0.31^{a}	363
Group-B	11±0.42^{c}	21±0.12^{d}	5±0.32^{d}	0.1±0.12^{d}	0^{d}	37.1
Group-C	13±0.13^{c}	40±0.32^{d}	21±0.17^{b}	8±0.29^{b}	0.8±0.13^{b}	82.8
Group-D	22±0.21^{b}	50±0.26^{b}	25±0.22^{b}	11±0.11^{b}	0.5±0.11^{b}	108.5
Group-E	17±0.33^{b}	35±0.21^{c}	18±0.35^{bc}	5±0.15^{c}	0.3±0.21^{b}	75.3
Group-F	11±0.27^{b}	28±0.31^{c}	14±0.13^{bc}	3±0.12^{c}	0.2±0.34^{bc}	56.2
Group-G	14±0.22^{bc}	24±0.32^{d}	11±0.43^{cd}	1.5±0.12^{c}	0.2±0.32^{bc}	50.7
Group-H	-	-	-	-	-	-
Group-I	-	-	-	-	-	-

Table 4: Influence of herbs on blood hematology of broiler chickens challenged with coccidiosis.

Groups	Blood hematology			
	RBCs (×10^{12}/ l)	Hemoglobin (g/dl)	WBCs (×10^{9}/l)	PCV (%)
Group-A	2.31^{cd}	7.20^{d}	115.31^{e}	53.00^{cd}
Group-B	2.61^{cd}	9.90^{abc}	119.61^{cd}	54.75^{c}
Group-C	2.67^{bc}	10.30^{bc}	120.67^{c}	55.01^{bc}
Group-D	2.81^{bc}	10.50^{abc}	122.81^{bc}	55.75^{b}
Group-E	2.73^{bc}	10.30^{abc}	122.73^{abc}	55.30^{bc}
Group-F	3.11^{ab}	10.67^{ab}	126.11^{ab}	56.10^{ab}
Group-G	2.90^{bc}	10.80^{ab}	124.90^{b}	56.40^{ab}
Group-H	3.41^{ab}	11.30^{a}	127.41^{a}	58.30^{a}
Group-I	3.80^{a}	11.53^{a}	130.80^{a}	59.15^{a}

Table 5: Influence of herbs on intestinal histomorphology of broiler chickens challenged with coccidiosis

Groups	Intestinal histomorphology Villi height measurement (µm)		
	Duodenum	Jejunum	Ileum
Group-A	1050^{e}	1037^{e}	990^{e}
Group-B	1090^{d}	1150^{d}	1140^{d}
Group-C	1110^{d}	1200^{cd}	1180^{cd}
Group-D	1155^{bc}	1218^{bc}	1200^{bcd}
Group-E	1160^{bc}	1220^{bc}	1211^{bcd}
Group-F	1165^{b}	1242^{bc}	1230^{bc}
Group-G	1171^{b}	1260^{ab}	1265^{ab}
Group-H	1198^{a}	1267^{a}	1287^{a}
Group-I	12.03^{a}	1280^{a}	1310^{a}

Group A = Control, Group-B = Aloe vera, Group-C = Giloy/Satgullo, Group-D = Tabashir, Group-E = Anwara, Group-F = Vaghayani/Hing, Group-G = Tamarind, Group-H = Compound-I, Group-I = Compound-II. $^{a-f}$ Superscripts with different letters showing significant level (P<0.05).

Intestinal histomorphology: Influence of different herbs on the villi height of the broiler chickens challenged with coccidiosis (Table 5). It has been reported that group H and I had significantly (P<0.05) better results as compared to other groups, whereas in groups A and B, the size of villi decreased, respectively.

Validation of immunity-related TLRs in PCR and qPCR: The above results of health and immunity in coccidiosis challenged broiler chickens were observed better in compound-I and II. Therefore, we have selected these two groups to evaluate the expression of immunity-related TLRs genes. The validation of TLRs was done on

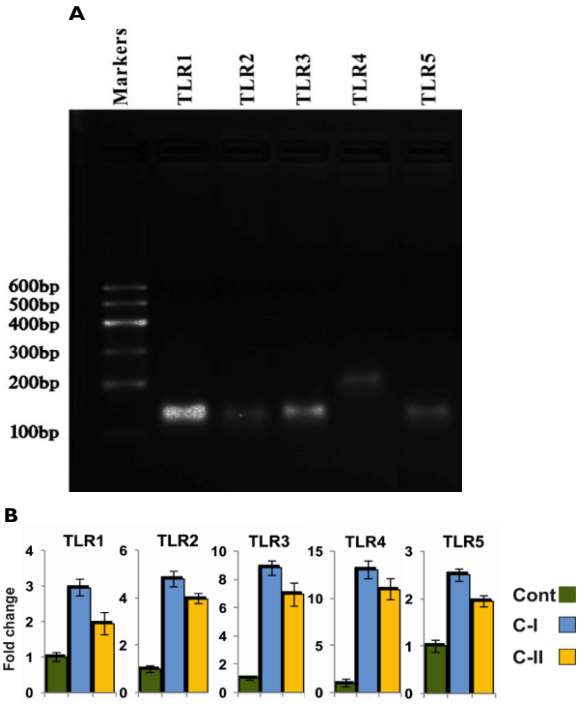

Fig. 3: Validation of immunity-related TLRs genes in PCR and qPCR of coccidiosis challenged broiler chickens: A=PCR, B=qPCR: Cont = coccidiosis challenged control without supplementation, CI= herbs supplementation without vitamin-C compound-I, CII = herbs supplementation with vitamin-C compound-II. TLR1- toll-like receptor-1, TLR2- toll-like receptor-2, TLR3- toll-like receptor-3, TLR4- toll-like receptor-4, TLR5- toll-like receptor-5.

PCR and qPCR. We have validated TLRs (1, 2, 3, 4 and 5), expressed in the erythrocytes of coccidiosis, in challenged broiler chicken (Fig. 3A).

The gene expression level related to immunity on coccidiosis-infected chicken erythrocytes were detected by real-time PCR. The results showed that TLR1, TLR2, TLR3, TLR4 and TLR5 were significantly upregulated in the coccidiosis challenged broiler chickens (P<0.05). All validations are consistent with the analytical results in this study (Fig. 3B).

DISCUSSION

Herbs are being investigated on a large scale worldwide. Our laboratory has done several works on this and found beneficial herbs/plants which have therapeutic usage and also can help to increase production (Rajput *et al*., 2012; Rajput *et al*., 2013; Jahejo *et al*., 2019). In this study, we have used different herbs/plants to investigate their effects on the health of coccidiosis challenged broiler chickens. Furthermore, we have investigated the regulatory effect of herbs on the expressions of TLRs immune-related genes.

Supplementation of *Azadirachta indica* and *Nicotiana tabacum*, flowers of *Calotropis procera plants* and seeds of *Trachyspermum ammi*plants and *A. absinthium* medicinal herbsagainst coccidiosis *(Eimeria tenella)* infection in broiler chicken significantly reduced bloody diarrhoea, improved weight gains and feed conversion ratio(FCR) similar to present results (Khan, 2012; Kostadinovic *et al*., 2012; Hady and Zaki, 2012). Moreover, Drăgan *et al*. (2014) reported a significant reduction in fecal oocysts, bloody diarrhea, and lesion score in *E. tenella* challenged broiler supplemented with *A. annua*, *Foeniculum vulgar* herbs.

According to Dragen *et al*. (2014), garlic plant extract administration increased the serum albumin, globulin, and total protein level due to garlic anti-inflammatory and immuno-modulatory action that refurbish the intestinal lesions produced due to *Eimeria* infection. However, in the present investigation, the low oocyst excretion rate, higher percentage of survival of infected birds, negligible signs of bloody diarrhea, minimum shedding of oocyst, and higher percent protection against caecal and intestinal lesions in medicinal plants supplemented groups of broiler chicken challenged with coccidiosis infection compared to control group of birds suggested the participation of some immune effectors present in the medicinal plants/herbs in shape of the tannins, phenols and phenolic acids, alkaloids, flavones, flavonoids, and flavonols is probably due to their ability to complex with extracellular and soluble proteins and to complex with parasite cell walls, more lipophilic flavonoids may also disrupt parasitic cell membranes that might inhibit the development of the parasites life cycle in the host (Kaleem *et al*., 2014). Moreover, during coccidial infection, the cytokine metabolite environment, produced within the microenvironment of the bird's intestine, may lead to physiological alterations including vasodilation, which caused increased hemorrhagic lesions in severely infected negative control chickens (Habibi *et al*., 2014).

The shape and integrity of the intestinal tract are important in maintaining the homeostasis of intestinal microbes, preventing infection, and promoting the digestion and absorption of nutrients (Gomes *et al*., 2014). In line with present findings, dietary cricket chitosan, and shrimp chitosan, medicinal herbs in basal diet improved intestinal villus height and reduced crypt depth with improving the body weight in the broiler (Ibitoye *et al*., 2019). Rajput *et al*. (2013) reported that the curcumin herb supplementation significantly increased thyroid hormone levels, which might be responsible for the increased WBCs and antibody production at the end of the trial.

Supplementation of *Aloe vera* and neem plant extracts in water reduced the number of gut *E. coli* populations similar to the current results (Darabighane *et al*., 2017). The application of plant extracts with basal diet considerably increased the numbers of *lactobacilli* bacteria in the ileum and caeca region of birds. The high levels of Acacia and Undaria plant extracts significantly reduced the number of pathogenic bacteria in the ileum and caeca compared and enhanced the activity of desirable microbiota (Vidanarachchi *et al*., 2010).

In the current study, the higher concentration of thyroid hormones because of herb supplementation stimulates the biosynthetic activity of the thyroid gland in birds, where the concentration of thyroid hormones significantly increased after herb supplementation with basal diet in the broiler (Rajput *et al*., 2013). T4 is more important for growth. Studies have shown that changes in plasma concentrations of T4 and T3 are related to growth. However, the absolute value of T4 is higher than the level of T3.

This study stated that chicken erythrocytes could respond to bacterial, fungi, parasites, and viral through the repertoire of TLRs expressed. TLR6 and TLR2 expression were significantly upregulated on coccidiosis infection in chicken erythrocytes. The chicken erythrocytes may have a role in immunity to coccidiosis because it has been shown that they could delay disease onset and reduce MDV genome copy number in the spleens of infected chickens (Parvizi *et al*., 2014; Abbas *et al*., 2017). It is probably because triggering the TLR4-associated pathways promotes type I IFNs genes' expression, which is important in the process of antiviral in chicken (TSekellick *et al*., 1998; akeda *et al*., 2003).

In contrast with current findings, of Rajput *et al*. (2013) reported that the curcumin supplementation significantly reduced the activity of ALT and AST. Carotenoids can promote aflatoxin-stimulated ALT and AST activity. In addition, free cholesterol levels are positively correlated with plasma ALT and AST levels (Cheng *et al*., 2001). Kaur *et al*. (2006) suggested that the herbal supplementation in infection-induced recipients may reduce the serum ALT, AST, and alkaline phosphate levels.

Conclusions: In this study, it was evaluated that the health and immunity of coccidiosis challenged broiler chickens improved with supplementation *of Aloe barbadensis*, *Tinaspora Cordifolia*. *Bambusaarundinacea*. The best result of this experiment was obtained when supplemented the mixture of *Aloe barbadensis*, *Tinaspora Cordifolia*, *Bambusaarundinacea*, *Emblica officinalis*, *Ferulafoetida regal*, and *Tamarindusindica* at the dose of 2ml/L. Moreover, we found these herbs are the regulator of immunity-related TLRs genes. Future studies be conducted to find the effects of these herbs alone or in combination with *Ocimum basilicum* on the growth performance and TLRs gene expression of broiler chickens challenged by heat stress/coccidiosis.

Authors contributions: NR designed the experiment. Data collection and experimental work by AAM. MN and AHS collected and analyzed the data, AAM and ARJ wrote the article. Final draft was approved by NR.

REFERENCES

Abbas A, Abbas RZ, Khan MK, *et al*., 2019. Anticoccidial effects of Trachyspermum ammi (Ajwain) in broiler chickens. Pak Vet J 39:301-4.

Abbas RZ, Abbas A, Raza MA, *et al*., 2019. In vitro anticoccidial activity of Trachyspermum ammi (Ajwain) extract on oocysts of Eimeria species of Chicken. Adv Life Sci 7:44-7.

Abbas A, Iqbal Z, Abbas RZ, *et al*., 2017. Immunomodulatory activity of Pinus radiata extract against coccidiosis in broiler chicken. Pak Vet J 37:145-9.

Ali H, Naqvi F, Tariq N, *et al*., 2014. Prevalence of coccidiosis and its association with risk factors in poultry of Quetta, Pakistan. Asian J App Sci 2:554-8.

Aroche R, Yordan M, Zheng R, *et al*., 2018. Dietary Inclusion of a Mixed Powder of Medicinal Plant Leaves Enhances the Feed Efficiency and Immune Function in Broiler Chickens. J Chem 5-27.

Cheng YH, Tian FS, Victor FP, *et al*., 2001. Effects of aflatoxin and carotenoids on growth performance and immune response in mule ducklings. Toxicol Pharmacol 128:19-26.

Darabighane B, Mirzaei AG, Navidshad B,*et al*., 2017. Effects of peppermint (Mentha piperita) and Aloe vera (Aloe barbadensis) on ileum microflora population and growth performance of broiler chickens in comparison with antibiotic growth promoter. Iranian J App Animal Sci 7:101-8.

Dragan L, Adriana G, Jorge FS, Ioan AP, *et al*., 2014. Effects of Artemisia annua and Foeniculum vulgare on chickens highly infected with Eimeria tenella (Phylum Apicomplexa). Acta Vet Scand 56:22.

Gerhold R, 2015. Overview of coccidiosis in poultry. Merck Manual-Veterinary Manual. 10th ed. Merck & Co., Inc., Kenilworth, NJ, USA. Accessed Oct. 2018.

Gomes AV, Quinteiro WM, Ribeiro A, *et al*., 2014. Overcrowding stress decreases macrophage activity and increases Salmonella Enteritidis invasion in broiler chickens. Avian Pathol 43:82-90.

Habibi H, Firouzi S, Nili S, Razavi M, 2014. Anticoccidial effects of herbal extracts on Eimeria tenella infection in broiler chickens: in vitro and in vivo study. J Parasitol Dis 2:401-7.

Hady MM and Zaki MM, 2012. Efficacy of some herbal feed additives on performance and control of cecal coccidiosis in broilers. Apcbee Procedia 4:163-8.

Hajati H, Ahmad H and Frahang A, 2014. Application of Medicinal Plants in Poultry Nutrition. J Med Plants and By-Products 1:1-12.

Hussain K, Iqbal Z, Abbas RZ, *et al*., 2017. Immunomodulatory activity of Glycyrrhiza glabra extract against mixed Eimeria infection in chickens. Int J Agric Biol 19:928-32.

Ibitoye EB, Lokman IH, Hezmee MN,*et al*., 2019. Gut health and serum growth hormone levels of broiler chickens fed dietary chitin and chitosan from cricket and shrimp. Poultry Sci 98: 745-52.

Jahejo AR, Jia FJ.Abbas RS, *et al*., 2020. Screening of Toll-like receptor signaling pathway-related genes and the response of recombinant glutathione S-transferase A3 protein to thiram induced apoptosis in chicken erythrocytes. Dev Comp Immunol pp:103-83.

Idris M, Abbas RZ, Masood S, *et al*., 2017. The potential of antioxidant rich essential oils against avian coccidiosis. World's Poult Sci J 73:89-104.

Jahejo AR, Rajput N, Tian WX, *et al*., 2019.Immunomodulatory and Growth Promoting Effects of Basil (Ocimum basilicum) and Ascorbic Acid in Heat Stressed Broiler Chickens. Pakistan J Zool 51:801.

Jahejo AR, Sayyad AR, Jia FJ, *et al*., 2020.Integration of gene expression profile data to screen and verify immune-related genes of chicken erythrocytes involved in Marek's disease virus. Microbial Pathogenesis104454.

Jahejo AR, Imdad HL, Sethar A, *et al*., 2016. Effect of heat stress and ascorbic acid on gut morphology of broiler chicken. Sindh Uni Res J (Science Series) 48:201-5.

Kaleem Q, Akhtar M, Awais M, Saleem M, *et al*., 2014. Studies on Emblica officinalis derived tannins for their immuno-stimulatory and protective activities against coccidiosis in industrial broiler chickens. The Sci. World J 1:1-10.

Kaur G, Tirkey N,Bharrhan S, *et al*., 2006. Inhibition of oxidative stress and cytokine activity by curcumin in amelioration of endotoxin-induced experimental hepatoxicity in rodents. Clin Experi Immunol 145:313-21.

Khan M, 2012. Anticoccidial activity of herbal complex in broiler chickens challenged with Eimeria tenella. Parasitol 139:237-43.

Khater HF, Ziam H, Abbas A, *et al*., 2020. Avian Coccidiosis: Recent advances in alternative control strategies and vaccine development. Agrobiol Rec 1:11-25.

Kostadinovic L, Jovanka L, Tamara GC, *et al*., 2012. Anticoccidian effects of the Artemisia absinthium L. extracts in broiler chickens. Archiva Zootechnica 15:69.

Morera D, Nerea R, Laia R, *et al*., 2011. RNA-Seq reveals an integrated immune response in nucleated erythrocytes. PloS one. 6: 26998.

Moryani AA, Rajput N, Naeem M and Shah AH, 2020. Prevalence of common poultry diseases in chicken and influence of different medicinal herbs on the growth of broiler chicken. Pure Appl Biol 9:1199-208.

Paolucci S, Neda B,Darren RW and Sharif S, 2013. Chicken erythrocytes respond to Toll-like receptor ligands by upregulating cytokine transcripts. Res Vet Sci 95:87-91.

Parvizi P, Mohamed FK, Amirul IM, *et al*., 2014. The effects of administration of ligands for Toll-like receptor 4 and 21 against Marek's disease in chickens. Vaccine 32:1932-8.

Passantino L, Massaro MA,Jirillo F, *et al*., 2007. Antigenically activated avian erythrocytes release cytokine-like factors: a conserved phylogenetic function discovered in fish. Immunopharmacol Iimmunotoxicol 29:141-52.

Rajput N, Naeem M, Ali S, *et al*., 2014. The effect of dietary supplementation with the natural carotenoids curcumin and lutein on pigmentation, oxidative stability and quality of meat from broiler chickens affected by a coccidiosis challenge. British Poul Sci 55:501-9.

Rajput N,Naeem M,Ali S, *et al*., 2013. The effect of dietary supplementation with the natural carotenoids curcumin and lutein on broiler pigmentation and immunity. Poult Sci 92:1177-85.

Rajput N,Naeem M, Rui Y, *et al*., 2012. Effect of dietary supplementation of curcumin on growth performance, intestinal morphology and nutrients utilization of broiler chicks. The J Poult Sci 012.0065.

Salman M, Abbas RZ, Israr M *et al*., 2020. Repellent and acaricidal activity of essential oils and their components against Rhipicephalus ticks in cattle. Vet Parasitol 283:109178.

Sekellick M, John WL, Terri EO, and Philip IM. 1998. Chicken interferon types I and II enhance synergistically the antiviral state and nitric oxide secretion. J Inter Cyt Res 18:407-14.

Shaw A, Kenneth SM and John PB, 2012. Phytase supplementation in a reduced calcium and phosphorus diet fed to broilers undergoing an Eimeria challenge. The J Poult Sci 120228015l-1202280151.

Takeda K, Tsuneyasu K, and Shizuo A. 2003. Toll-like receptors. Ann Rev Immunol 21:335-76.

Tekeli A, Elik L, Kutlu HR, Grgulu M, *et al*., 2006. Effect of dietary supplemental plant extracts on performance, carcass characteristics, digestive system development, intestinal microflora and some blood parameters of broiler chicks. Abstract Book of 12th Eur Poult Conf, Verona- Italy 10-14th September, 2006.

Velkers F, Swinkels WJ, Annemarie R, *et al*., 2010. Effect of Eimeria acervulina infection history on the immune response and transmission in broilers. Vet Parasitol 173:184-92.

Vidanarachchi JK,Elangovan AV, Lene LM, *et al*., 2010. Effect of some plant extracts on growth performance, intestinal morphology, microflora composition and activity in broiler chickens. Ani Prod Sci 50:880-9.

Williams, R. 1998. Epidemiological aspects of the use of live anticoccidial vaccines for chickens. Int J Parasitol 28:1089-98.

Zhang K, Li X, Na C, *et al*., 2020. Anticoccidial effects of *Camellia sinensis* (green tea) extract and its effect on Blood and Serum chemistry of broiler chickens. Pak Vet J 40:77-80.

Zaman MA, Abbas RZ, Qamar Wm *et al*., 2020. Role of secondary metabolites of medicinal plants against *Ascaridia galli*. Worlds Poult Sci J 76:639-55.