ANTIBACTERIAL AND HISTO-PATHOLOGICAL EFFECTS OF Β-GLUCAN AND *LACTOBACILLUS* IN *ESCHERICHIA COLI* INFECTED BROILER CHICKS

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ABSTRACT: Prebiotics like β-glucan from wheat bran sources and *Lactobacillus* from yoghurt were used in broiler chickens against pathogenic *Escherichia coli*. A total of 120 broiler hatchlings were reared into 5 groups, A to E, with 24 birds in each group. Group A and Group B were taken as control negative and positive, respectively. The birds in group C were augmented with β-glucan at 75mg/Kg and *E. coli* infection. The birds of group D were nourished with *Lactobacillus* @10^9 CFU along with *E. coli* infection. Six birds from each group were bled on the 21st, 28th, 35th and 42nd days to observe pathological and hematological changes. The highest morbidity and mortality rates were 50% and 44% which were recorded in the control positive Group B while the lowest were 0% and 0% in the control negative group A. The highest weight gain was recorded in Group C, followed by E, D, A, and B.

Key words: β-glucan, Broilers, *E. coli*, Histopathological, *Lactobacillus*.

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INTRODUCTION

The poultry industry is facing many challenges in its progress in Pakistan. At present, the biggest challenge is to control the bacterial ailments in poultry. These bacterial diseases are meant for heavy economic losses. Major bacterial diseases of poultry are *colibacillosis, salmonellosis* and *Campylobacteriosis* which cause serious problems in the poultry industry with respect to the economics (Patterson and Burkholder 2013). *Escherichia coli* is responsible for the most prevalent contagious disease of poultry birds, named *colibacillosis*. The important recognized serotypes of *E. coli* are O78:K80 (B), O2:K2 (L), and O1:K1 (L). These strains are known as Avian Pathogenic *E. coli* (APEC). A high number of *E. coli* are present in the poultry droppings, the most common route is the inhalation of the fecally contaminated dust, which contains large numbers of avian pathogenic *E. coli*. Young birds usually die due to septicaemia (Janben *et al.,* 2011). *Colibacillosis* is cosmopolitan and is generally reported in the poultry industry every year with a mortality rate (6–55 %) and high morbidity rate (Margulies *et al.,* 2015). *E. coli* infects many systems of poultry birds, resulting in acute septicaemia, pericarditis, perihepatitis, enteritis, cellulitis, panophthalmitis, arthritis, ovarian follicle degeneration, and osteomalasia. Hens suffering from *colibacillosis* show signs of salpingitis leading to vertical transmission. A 0.3% to 3% mortality found in the early life of poultry birds when infected with *colibacillosis* (Borgmann *et al.*, 2013). Frequent use of antibiotics against bacterial diseases resulted in antibiotic resistance and drug residues in poultry meat. Antibiotic therapy may also increase the systemic complications such as hepatic syndrome and renal failure, so direction is bustling towards the usage of alternative means for treating infectious diseases. Probiotics (alive microorganisms) administered in sufficient amounts can eliminate the pathogenic organisms. These are non-digestible diet ingredients that stimulate the growth and activity of one or limited bacteria in the colon that improve the health of the organism. These are widely being used as alternative means that improve intestinal microflora and produce immunity against disease (Tarr *et al.,* 2018).

A lot of soluble fibers such as β-d-glucan (polysaccharide in nature), have shown effects on insulin production, cholesterol regulation, and controlling glycemia. Many cereals, e.g., oats, wheat, and barley, are rich sources of this useful constituent. Wheat harvest is predominant (15 million tons annually) in the UK than other cereals, and it has about 1% β-glucan (Fortier, 2018). β-glucan is used for diabetes, cancer, high cholesterol and HIV/AIDS patients. It is also utilized to enhance the immune response in people whose body defense systems have been weakened by physical and emotional stress, chronic fatigue, or treatments such as chemotherapy radiation. β-glucan has also been effectively used against flu (influenza), common cold, allergies, H1N1 (swine flu), Lyme disease, hepatitis, asthma, Crohn's disease, ear infections, aging, ulcerative colitis, rheumatoid arthritis, fibromyalgia, and multiple sclerosis. In the USA β-glucan is being used as an alternative to antibiotics (Sari *et al.,* 2017). Improvement in inherent immune response is initiated after the attachment of β-glucan to specific receptors (dectin-1) already present on the cell surface of dendritic and macrophages. As a result of this binding, the innate immune cells (B and T lymphocytes or natural killer a lot of interleukins (IL-4, IL-6) and tumor necrosis factor (TNF-α). Water-insoluble β-glucans have stronger immune-stimulating activity as compared to their water-soluble equivalents. β-glucans are also used as potent antifungal agents. These are also responsible for increasing the weight of vital organs. The number of WBCs also enhanced by β-glucans. These are also responsible for HI antibody titre against ND and IBD. β-glucans are also used as texturing agents in various nutraceutical and cosmetic products (Nouban *et al.,* 2016). β-glucan increases the activity of heterophil cells, macrophages and produces immune-modulatory effects (Chen *et al.,* 2018).

Among probiotics, the *Lactobacillus* (LAB) species are the most common, found in feed such as yogurt, cheese, beer, fermented food, etc. These are Gram-positive, rod shaped, and facultatively anaerobic. Normally, *Lactobacillus* species are a major part of gut and vaginal microbiota and produce biofilm. Over 180 spp. of *Lactobacillus* are present in the world. Their antifungal and antibacterial activity depends on the production of bacteriocins and low molecular weight compounds such as acetaldehyde, diethyl, and certain organic acids (Sarkar, 2016). *Lactobacillus* application's major purpose is to maintain human health and also be helpful for the treatment of vaginal infections, eczema, and diarrhea. *Lactobacillus* species also help to prevent infections, e.g., lung infections in children, vaginal infections, and skin problems like Acne, lactose intolerance, Lyme disease, and high cholesterol.

In poultry, *Lactobacillus* heat-killed strains like LAH7, LAP5, and LAF1 have the ability to adhere to GIT epithelium and protect the host from infectious. In return, the growth of *E. coli* and *salmonella* is arrested (Ni *et al.,* 2017). Other strains, such as LF33, also have the ability to adhere to intestinal cells and inhibit the activity of *E. coli*, *Staphylococcus aureus*, and *Salmonella typhimurium* (Aik *et al.,* 2018). It also helps host organisms against by promoting epithelial cell line integrity (Durmaz *et al.,* 2016). All available literature is about the separate use of *Lactobacillus* and β-glucan, which are effective against *E. coli* infection and the growth of broilers, but no data is available on the combined use of these useful constituents.

The current study aimed to find out the control measures and minimize loss due to E.coli infection in broiler industry and in this scenario to evaluate the antibacterial and Histo-pathological effects of β-Glucan and lactobacillus in *Escherichia coli* infected broiler chicks.

MATERIALS AND METHODS

Research design: The current study was conducted at veterinary and poultry research farms of Faculty of Veterinary and Animal Sciences, Gomal University Dera Ismail Khan. After statistical design and calculation a total of 120 day-old chicks having an initial weight range of (41.05±0.12) were purchased and distributed into 5 groups (A to E), each containing 24 birds. Feed and water were supplied at standard levels to all birds. Birds were vaccinated against Newcastle Disease (ND), Infectious Bronchitis (IB), Infectious Bursal Disease (IBD, D78, 228E) and Hydro Pericardium Syndromes (HPS) according to the recommended schedule.

Experimental plan: The chicks of Group A were kept as control negative and Group B as control positive while Group C was supplemented by β-glucan along with *E. coli* infection. Birds of Group D were fed on *Lactobacillus* and infected with *E. coli*. Birds of group E were collectively supplemented by β-glucan and *Lactobacillus* along with *E. coli* infection as shown in Table 1. Through the respiratory route, *E. coli* (10^6 CFU) infection was given at the age of 15^{th} day. β-glucan was fed at a dose rate of 75 mg/Kg (Moon *et al.,* 2016) along with *Lactobacillus* (10^9 CFU), which were mixed in water. After introducing the infection, six birds from each group were bled at every 7 days interval. Blood was taken aseptically to carry out hematological studies. Vital organs with pathological lesions were collected and preserved in 10% buffered formalin (Aziz-ur-Rehman *et al.,* 2017).

Pathological Studies: A total of 120 broiler birds were kept under observation for 4 weeks. Clinical signs, gross lesions, and organs weight were noticed. On the 21^{st}, 28^{th}, 35^{th}, and 42^{nd} days, 6 birds from each group were bled, and a complete pathological examination of inner organs was conducted. Moreover, the vital organs having prominent lesions were preserved in 10% buffered formalin for histopathological studies (Aziz-ur-Rehman *et al.,* 2017).

Haematological studies: Blood samples from all groups were collected in EDTA-soaked tubes, and hematological studies were conducted as described by Benjamin, 1978.

- Total erythrocyte counts (RBCs)
- Total leukocyte counts (TLC)
- Packed cell volume (PCV)

- Haemoglobin concentration (Hb) (Sahli's method)

Statistical analysis" Results are expressed as mean ± SE. The results obtained were analyzed and compared by one-way ANOVA followed by post hoc Tukey's adjustment using the Statistical Package for Social Sciences (SPSS, version 16, Inc, Chicago, Illinois, USA). P<0.05 was considered to be statistically significant. Data have been presented as mean and standard error of mean (SEM).

RESULTS

Body weight gain: A total 120 day-old chicks were purchased from a local hatchery having an initial weight range of (41.05±0.12). During the trial the birds were regularly monitored for any decrease or increase in the body weight of an individual bird of each group, as summarized in Fig: 1. The maximum weight gain was recorded in the birds of Group C and Group E (P<0.001) as compared to other groups as shown in Fig: 1.

Morbidity and Mortality percentage: From Fig: 2. it is clear that 0% mortality and morbidity was seen in the birds of Group A. The birds of group B indicated the highest percentage of mortality (40%) and morbidity (50%) due to *E. coli* infection as compared to all other groups. As Group C was the β-glucan-supplemented group along with *E. coli* infection. In this group, mortality and morbidity were significantly (P<0.001) lowered. Morbidity was 25%, and mortality was 12.5%, which is lesser than B. Group D was supplemented by *Lactobacillus* and challenged with *E. coli*. Morbidity rate was 29%, and mortality was 16.3%. In group E, combined supplementations of β-glucan and *Lactobacillus* have significant effects (P<0.001) to control the mortality rate. So similar to Group A, no mortality and 12.5% morbidity were observed.

Gross lesions on vital organs in the birds of various groups: Post-mortem examination was weekly conducted to examine the lesions of pathogenic *E. coli* and beneficial effects of supplementation.

Group A: As control negative birds were not exposed to infection, no post-mortem lesions were detected in this group.

Group B: Due to control, positive severe lesions were found on heart (pericarditis) and liver (perihepatitis) and on air sacs.

Group C: Trivial post-mortem lesions were seen in these birds. In this group only tracheitis and pericarditis were seen, and no perihepatitis was found.

Group D: In this group, mild to moderate lesions were shown. Pericarditis and perihepatitis were seen on the 7th and 14th days of post-infection.

Group E; No severe lesions were found in this group. Combined supplementation of β-glucan and *Lactobacillus* resulted in good effects on organs' weight as well as their performance. Good results were observed due to the use of probiotics and prebiotics as compared to all other groups, as indicated in Figs: 3 and 4.

Hematology

TLC count (10^3/ul): Total leukocytes were found to be the highest (P<0.001) in group E (β-glucan and *Lactobacillus*) followed by group C (β-glucan) (P<0.001) and group D (*Lactobacillus*) (P <0.001) as compared to control groups A & B throughout the trial period. High levels of WBCs were seen in group B due to *E. coli* infection as shown in Table 2.

TECs count (10^6/ul): Total erythrocyte count was lower (P<0.001) in group B as compared to all other groups throughout the trial, while it differed among the rest of the groups from the negative control (A). Decreased (P<0.001) RBCs were seen in Group C and D on the 32nd day while at 39th the day in Group E significant increase (P<0.001) of RBCs was seen as indicated in Table 2.

Haemoglobin Concentration (g/dl): Hemoglobin concentration was recorded as the lowest (P<0.001) in the *E. coli*-infected group B as compared to others. In all other groups, a very little difference was found in Hb concentration. Supplemented groups (D and E) showed some increase in hemoglobin concentration at the 25th day of age than group B while lower in group A throughout the experiment, as shown in Table 2.

Table 1. Different experimental groups of broiler birds along with supplement schedule.

Groups	No. of Birds	Treatment from15 days of age
A	24	Control –ve (*E.Coli* non-infected)
B	24	Control +ve (*E.coli* infected)
C	24	β-glucan and *E. coli*
D	24	*Lactobacillus* and *E. coli*
E	24	β-glucan+ *Lactobacillus*+ *E. coli*
Total	**120**	

Packed cell volume (PCV %): PCV was lower in group B as compared to others. On the 25th day, group D and E showed noteworthy differences as compared to other groups, while at 32nd day group E exhibited a higher difference from all other groups. At 39th day of age, group C, as followed by group D and E has a lower percentage of PCV as compared to the rest of the groups as indicated in Table 2.

Comparative increase in the weight gain of vital organs: Six birds from each group were bled on weekly basis and post-mortem was conducted to measure the change in the weight of vital organs of an individual bird of respective group. The maximum increase was noticed in the weight of vital organs in Group A and Group E as compared to other groups.

Table 2: Comparative haematological parameters in *Escherichia coli* infected broiler chicks of various groups fed on β-glucan and *Lactobacillus.*

DPI	Properties	G-A	G-B	G-C	G-D	G-E
25th	WBCs	21.54± 1.14a	23.94 ± 0.59a	22.13±0.70a	21.23±0.85a	23.53±2.44a
	RBCs	2.576±0.281a	2.33±1.96b	2.43±3.22b	2.35±4.47b	2.57±4.45a
	PCV	2.576±0.281a	2.33±1.96b	2.43±3.22b	2.35±4.47b	2.57±4.45a
	HB	11.8±4.5a	10.33±1.96b	12.14±3.22a	13.07±4.47a	12.87±4.45a
32nd	WBCs	23.59 ± 1.8a	28.49±0.95ab	25.97±0.66a	22.02 ±1.62a	25.68±3.05a
	RBCs	2.625±0.223a	2.40±1.51b	2.51±4.76a	2.43±4.78b	2.61±4.34a
	PCV	2.625±0.223a	2.40±1.51b	2.51±4.76a	2.43±4.78b	2.61±4.34a
	HB	13.25±2.06a	11.50±1.51b	13.00±4.76a	12.57±4.78b	13.25±4.34a
39th	WBCs	24.50±0.65b	32.49 ± 0.28a	26.13±0.72b	25.26± 2.52b	26.73±1.71b
	RBCs	2.68±0.12a	2.22±1.96b	2.58±2.33a	2.25±4.47b	2.70±4.34a
	PCV	2.68±0.12a	2.22±1.96b	2.58±2.33a	2.25±4.47b	2.70±4.34a
	HB	13.33±2.33a	11.22±1.96b	13.33±2.33a	12.85±4.47a	13.14±4.34a

Mean ± SD having similar alphabets in a row are statistically non-significant (P>0.05)
A= No Infection (Control -ve)
B= Infection with *E. coli* (Control +ve)
C= β-glucan @ 75mg/Kg +*E. coli* Infection
D= *Lactobacillus* @ 109 CFU+ *E. coli* Infection
E= Combination of β-glucan and *Lactobacillus* + *E. coli* Infection

Table 3: Comparative Increase in the weight (gm) of vital organs in *Escherichia coli* infected broiler chicks fed on β-glucan and *Lactobacillus*.

Weeks	Organs	Group A	Group B	Group C	Group D	Group E
25thday	Liver	5.96 ± 1.47^{a}	4.80 ± 0.161^{c}	5.371 ± 0.712^{b}	5.124 ± 0.27^{b}	5.97 ± 0.517^{a}
	Heart	5.96 ± 1.47^{a}	4.80 ± 0.16^{c}	5.371 ± 0.712^{b}	5.124 ± 0.27^{b}	5.97 ± 0.517^{a}
	Lung	41.55 ± 1.47^{a}	38.65 ± 0.16^{b}	42.71 ± 0.712^{a}	42.12 ± 0.27^{a}	37.97 ± 0.57^{a}
32ndday	Liver	7.92 ± 2.25^{a}	5.53 ± 1.61^{c}	6.32 ± 0.48^{b}	6.907 ± 0.34^{b}	7.451 ± 1.18^{ab}
	Heart	7.92 ± 2.25^{a}	5.53 ± 1.61^{c}	6.32 ± 0.48^{b}	5.907 ± 0.35^{a}	7.451 ± 1.18^{ab}
	Lung	43.77 ± 2.25^{a}	35.63 ± 1.61^{c}	42.57 ± 0.48^{ab}	39.90 ± 0.35^{b}	43.45 ± 1.18^{ab}
39thday	Liver	9.124 ± 1.286^{a}	7.77 ± 1.427^{ab}	7.91 ± 1.396^{ab}	8.692 ± 0.76^{b}	8.795 ± 1.247^{a}
	Heart	9.124 ± 1.286^{a}	7.77 ± 1.427^{ab}	7.91 ± 1.396^{ab}	8.692 ± 0.76^{b}	9.795 ± 1.247^{a}
	Lung	45.59 ± 1.286^{a}	39.88 ± 1.427^{b}	44.88 ± 1.396^{ab}	41.69 ± 0.76^{b}	46.79 ± 1.247^{a}

Mean ± SD having similar alphabets in a row are statistically non-significant (P>0.05)

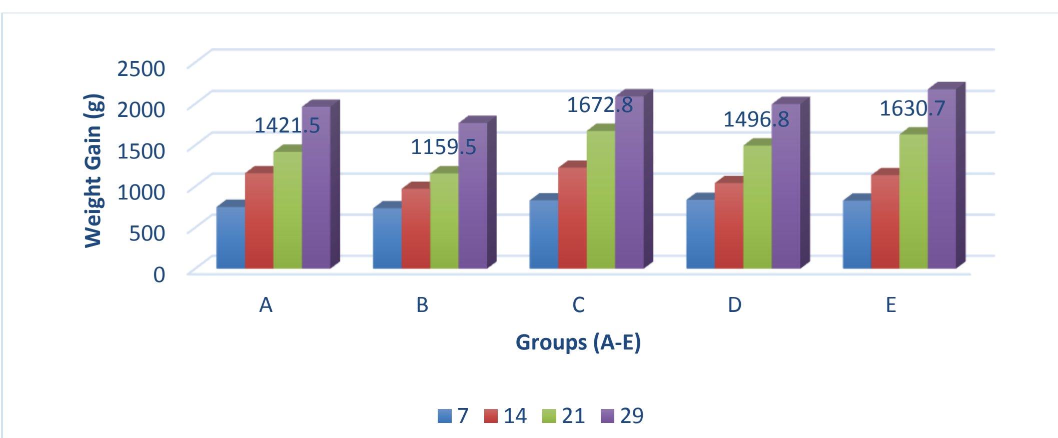

Fig 1: Graph showing comparative weight differences increase in various experimental broiler groups

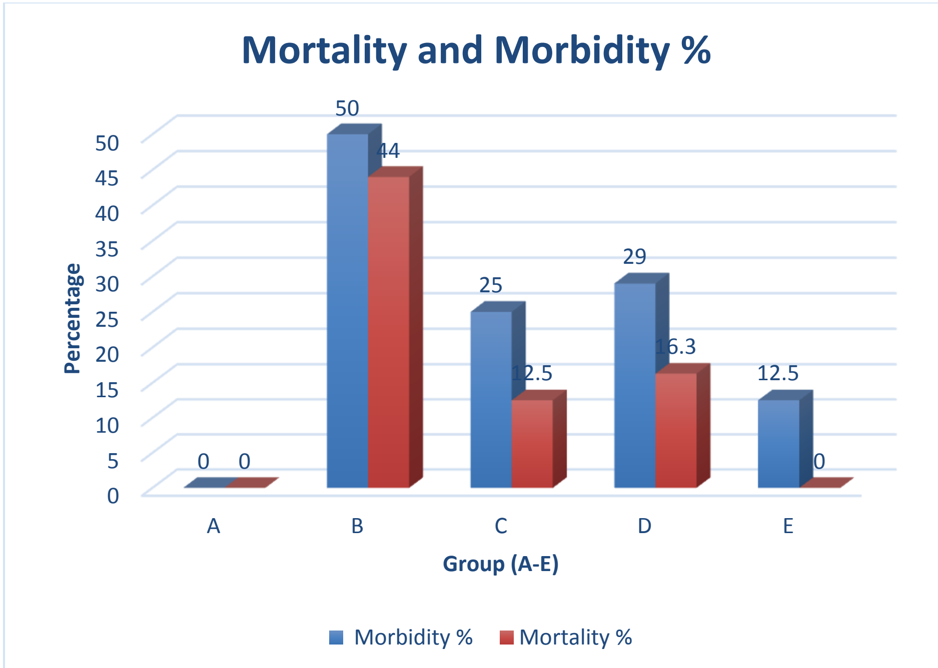

Fig 2: A Graph of Mortality and Morbidity percentage in different broiler birds.

Fig 3: Combined use of β-glucan and *Lactobacillus* + *E. coli* Infection

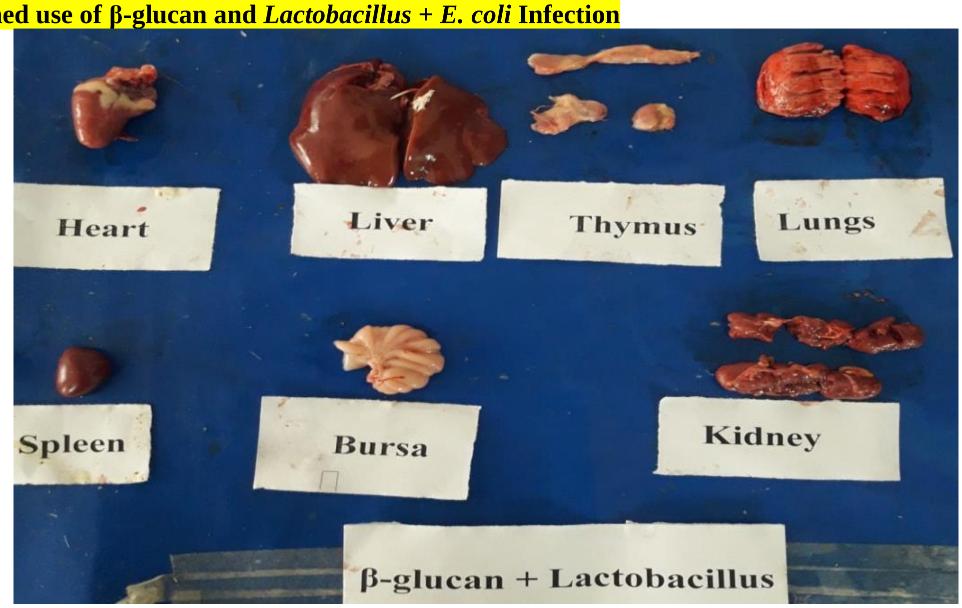

Fig: 4 Lesions in Group B, Group C and Group D

DISCUSSION

A total of 40-45% of meat requirements are being fulfilled through poultry products. Therefore, to meet the baseline requirement of meat and eggs, it is necessary to develop the poultry industry (Rehman *et al.*, 2020). In poultry, frequent uses of therapeutic antibiotics to prevent infectious diseases have led to the problem of antibiotic resistance and drug residues. Prebiotics such as β-glucan have been proven to have special effects on insulin production, managing cholesterol, and managing glycemia (Landis *et al.*, 2020). β-glucan is being used as an alternative to antibiotics (Volman and Bazhenov, 2019). *Lactobacillus* species' antifungal and antibacterial activity depends on the production of bacteriocins and low molecular weight compounds such as acetaldehyde, diethyl, and certain organic acids (Sarkar, 2016). In poultry, *Lactobacillus* heat-killed strains like LAH7, LAP5, and LAF1 have the ability to adhere to GIT epithelium and protect the host from infectious microorganism (Wang *et al.,* 2007).

Low mortality and morbidity rates were seen in all of *Lactobacillus*-fed birds compared to the birds of the control groups, as indicated in Fig: 2. The same findings were seen by Yousef (Yousef, 2017) who found that the supplementation of 40 mg/kg of *Lactobacillus* decreased mortality and increased the body weight up to 8-10%, as well as improved as GIT microflora was also improved. Feed intake and body weight of the β-glucan (75 mg/kg) supplemented group were higher as compared to *E. coli*-infected groups from 3 to 6 weeks of age. The β-glucan has the ability to control *E. coli* bacteria by providing immunity that is similar to previous results (Lv *et al.,* 2017). The body weight of chickens was significantly higher in *Lactobacillus*-supplemented groups as shown in Fig: 1. Similar findings were recorded by Dawood (Dawood *et al.,* 2020) that there was a significant increase in the body weight of broiler birds due to supplementation of *Lactobacillus.* The mortality and morbidity rates were prominently less in β-glucan-fed birds as compared to the birds of the control groups as indicated by Adigun (Adigun *et al.,* 2010).

A significant increase was recorded in the absolute and relative weight of the liver and heart in the birds of group D than in then rest of the groups, as indicated in Table 3, which are in accordance with the finding that the liver was significantly increased in the treated group as compared to the control (Adigun *et al*., 2010). The major fighting cells of the body are WBCs, which are normally increased in any disease condition, so in the present study in the *E coli*-infected group, these were found higher, as well as in group D, which were supplemented with the *Lactobacillus*; these were found higher than the control negative group (Table 2) as Dong *et al*. (Dong *et al.,* 2013) reported that the WBCs count was significantly increased from 4.08 to 4.55 after the *Lactobacillus* treatment (P-0.05). Hemoglobin concentration (Hb) was decreased in *Lactobacillus*-supplemented birds as described by Antonio *et al*. (Antonio *et al.,* 2018).

Severe pericarditis and severe perihepatitis were observed in the positive control group as shown in Fig: 4. The control positive group was infected with *E. coli.* While in group A (non-infected), which was control negative, no lesions were observed. The same findings were seen by Bianchini *et al*. (Bianchini *et al.,* 2020) who found that there was air saculitis, perihepatitis, and pericarditis in broiler chickens that were infected with *E. coli*. The combined use of probiotics (*Lactobacillus*) and prebiotics (β-glucan) has a good effect. As Safaei *et al*. (Safaei *et al.,* 2019) evaluated the effect of probiotics in combination with β-glucan. The β-glucan at @0.25%, 0.5%, and .1% was used. The probiotics (*Bacillus amyloliquefaciens*) were fed to broilers at dose rates of 0.05%, 0.1%, and 0.2%. The total duration of this experimental study was 5 weeks. Growth rate and FCR increased by β-glucan and BA-Pro fed groups as compared to the control group. Al-Dholin *et al.* (2009) reported that the values of Hb, ESR, RBCs, WBCs, PCVs, and total Igs were also significantly higher in fish fed with *Lactobacillus*-supplemented diet than in the control group. In this trial it was noticed that β-glucan-supplemented birds showed increased weight gain and feed intake and performance. Similarly, Moran *et al*. (Moran *et al.,* 2012) observed β-glucan effects on growth and immune responses in broilers. The B-glucan has shown better effects on broiler performance (Teng *et al.,* 2021; Wang *et al.,* 2021). Lactobacilli showed improvement in intestinal function (Zaghari *et al.,* 2020). Bilal *et al.* (2021) studied the active role of yeast in broilers.

In this study, β-glucan-supplemented groups showed antibacterial activity because it boosted up the immune system of birds. Similarly, Mantovani (Mantovani, 2019) investigated the different effects of β-glucan derived from oat, barley, and mushroom cell walls. The β-glucan stimulates humoral and cellular immunity by stimulating macrophages, natural killer and T helper cells, and activation of T lymphocytes. β-glucan improved growth by stimulating TSR and CD8 receptors. Oat-derived β-glucan showed antibacterial activity against *E coli* and *Bacillus subtilis.* During the trial it was noticed that *E. coli*-infected birds were not active for the whole length of time. These were depressed and less attracted towards feed. In Group B, *E. coli*-infected broiler chickens showed lesions of pericarditis, perihepatitis, and airsaculitis. The same findings were seen by Gryspeerdt *et al*. (Gryspeerdt *et al.,* 2018) who observed the *E coli* lesion of pericarditis, perihepatitis, and airsaculitis.

Conclusion: The present study concludes that β-glucan and *Lactobacillus* have the ability to lower the mortality and morbidity rate during experimental *E. coli* infection. Broiler chickens treated with β-glucan and Lactobacillus showed mild lesions and fewer clinical signs as compared to untreated chickens and also can be used to increase the body weight and performance of broilers. It was recorded that β-glucan and *Lactobacillus* can be used during *E. coli* infection to decrease economic losses.

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