Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2023.013

RESEARCH ARTICLE

Purification, Characterization and Protective Effects of Indigenous Yeast Derived β-glucans against Salmonellosis in Broilers

Muhammad Irfan Anwar[1*,] Muhammad Ali Tahir[1], Mian Muhammad Awais[1], Ahmad Raza[1,2], Masood Akhtar[1], Faqir Muhammad[2], Muhammad Raza Hameed[1] and Nabeel Ijaz[3]

[1]Department of Pathobiology, Faculty of Veterinary Sciences, Bahauddin Zakariya University, Multan, Pakistan
[2]Department of Biosciences, Faculty of Veterinary Sciences, Bahauddin Zakariya University, Multan, Pakistan
[3]Department of Clinical Sciences, Faculty of Veterinary Sciences, Bahauddin Zakariya University, Multan, Pakistan
*Corresponding author: drmirfananwar@gmail.com; drirfananwar@hotmail.com

ARTICLE HISTORY (22-242)

Received: July 18, 2022
Revised: January 27, 2023
Accepted: January 31, 2023
Published online: February 12, 2023

Key words:
Saccharomyces cerevisiae
β-glucans
Salmonella typhimurium
Antibiotic alternative
Immunomodulator

A B S T R A C T

Indigenous *Saccharomyces* (*S.*) *cerevisiae* was cultured under submerged fermentation conditions and β-glucans were extracted by enzymatic method. The extracted β-glucans were characterized by FT-IR and their spectra presented peaks at a wavelength of 1021 cm^{-1}. Safety and mutagenicity testing of β-glucans showed them non-mutagenic and non-toxic up to a concentration of 200 mg/mL. For immunotherapeutic effects against *Salmonella* (*Sal.*) *typhimurium* infection, broiler chicks (n=120) were randomly divided into 4 equal groups viz. A (negative control), B (positive control challenged with *Sal. typhimurium*; 1.0×10^{10} cfu), C (β-glucans at the dose rate of 50 mg/kg feed + *Sal. typhimurium* challenge), and D (β-glucans at the dose rate of 100 mg/kg feed + *Sal. typhimurium* challenge). Results revealed protective effects of β-glucans against *Sal. typhimurium* infection as depicted by mild clinical signs along with minimal/no gross and histopathological lesions. Immunological responses were also higher ($P<0.05$) in β-glucans administered birds. β-glucans also modulated intestinal morphology with improved villi-height and crypt-depth ratio. In conclusion, β-glucans isolated from indigenous *S. cerevisiae* conferred protective effects against salmonellosis and therefore can be used successfully in broilers.

To Cite This Article: Anwar MI, Tahir MA, Awais MM, Raza A, Akhtar M, Muhammad F, Hameed MR and Ijaz N, 2023. Purification, characterization and protective effects of indigenous yeast derived β-glucans against salmonellosis in broilers. Pak Vet J, 43(2): 297-302. http://dx.doi.org/10.29261/pakvetj/2023.013

INTRODUCTION

Chicken meat and eggs are the most important sources of high-quality protein for the many millions of people who live in poverty (Farrell, 2013). This makes the poultry industry an essential component of food chain but microbial challenges especially enteric pathogens in commercial poultry cause significant economic losses to this industry (Hofmeyr, 2019). Vaccination may be the most efficient method for preventing illnesses with a single causative agent. Antibiotics may be preferable over vaccination when a range of infectious pathogens and environmental factors have a role in causing the illnesses. However, the emergence of resistant in bacterial population against available antibiotics is a big issue which has markedly increased the incidence of bacterial invasion in organs and body tissues of poultry birds with subsequent foodborne illnesses in consumers (Suresh *et al*., 2018). Among these, *Salmonella* (*Sal.*) *typhimurium* is one of the primary causes of food-borne gastroenteritis in people and is frequently linked to the consumption of chicken meat and eggs that are infected with *Salmonella* (Shao *et al*., 2013). Salmonellosis has been prevented in poultry production through a variety of preventative measures, such as improved hygiene standards, use of antibiotics, immunization by *Salmonella* vaccines, use of competitive exclusion products and probiotics, in addition to genetic selection of chicken lines for stronger immune responses (Collett *et al*., 2020). The development of antibiotic resistance by pathogenic bacteria has prompted a global discussion on reducing the use of antibiotics in poultry production, even though medicines are one of the most effective controls for *Sal. typhimurium* infection. Therefore, finding new solutions to bacterial problems in industrial chicken production is crucial.

Yeast-derived products such as β-glucans are extensively used in the poultry industry. These have anti-microbial and immunomodulatory properties which enable the poultry birds to fight against the invading microorganisms without exerting any negative impact on

their production performance (Ding *et al*., 2019; Schwartz and Vetvicka, 2021). Yeast β-glucans consist of β-1, 3/1, 6 glycoside linkages and have a strong affinity to bind with receptors of different immune cells which are helpful in activating lymphocytic production, cytokines, chemokines, microbial killing and to improve the development and integrity of the intestine (Lei, 2019; Amer *et al*., 2022). The structural integrity of β-glucans play important role in those functions which depend on extraction and purification methods (Han *et al*., 2020). Usually, extraction of β-glucans from *Saccharomyces* (*S*.) *cerevisiae* cell involve alkali or acids washing or a combination of both, which solubilize proteins and other polysaccharides that leads to a more or less strong degradation of the glucose chains/ glucan structure (Liu *et al*., 2008). Therefore, extraction and purification of β-glucans with less aggressive methods to maintain native structure are required to attain desired biological effects.

The current study was designed to purify and characterize the yeast-derived β-glucans from indigenous yeast cultures with their *in vitro* evaluation of cytotoxicity and mutagenicity before their application in poultry feed. Further, protective and immune-modulating effects of these yeast-derived β-glucans against *Sal. typhimurium* in industrial broilers have also been observed in this study.

MATERIALS AND METHODS

Collection, isolation and identification of *S. cerevisiae*: The *S. cerevisiae* was cultured on yeast peptone dextrose agar from three different sources including baker's yeast, sugar cane sludge and molasses by following the methodology described previously (Rezaei *et al*., 2014).

Extraction, purification and quantification of β-glucans derived from indigenous *S. cerevisiae*: The yeast cells were autolyzed according to the methodology described by Liu *et al*. (2008) followed by treatment with hot water and enzymes according to the methodologies of Borchani *et al*. (2016) and Freimund *et al*. (2003). The purified β-glucans were quantified by Yeast β-glucan assay kit (Megazyme®, USA).

Molecular characterization of β-glucans using Fourier transform infrared (FT-IR) spectroscopy: FT-IR spectroscopy was used for the molecular characterization of β-glucans by following the methodology described by Zechner-Krpan *et al*. (2010).

Safety testing of isolated β-glucans: The cytotoxic effects were enumerated in terms of percent hemolysis by graded doses of β-glucans in comparison with Triton-X (positive control) by using the methodology described by Abd Razak *et al*. (2007). The mutagenic effects were determined by the Ames test as described by Halwani *et al*. (2015).

Collection of infective material: The intestinal contents of broilers suspected for salmonellosis were collected from the University Diagnostic Laboratory, Department of Pathobiology, Bahauddin Zakariya University (BZU), Multan, Pakistan. The gut contents were streaked on Xylose Lysine Deoxycholate agar (XLD) for culturing and isolation of *Sal. typhimurium.* The identification of *Sal. typhimurium* was done based on colony morphology on XLD and biochemical characterization as described by Tarabees *et al*. (2017).

Experimental design: One-day-old broiler chicks (n=120; Cobb) were procured from a local hatchery and after acclimatization period, the birds were randomly divided into 4 equal groups and administered with different treatments as follows:

Group A = negative control
Group B = Positive control challenged with *Sal. typhimurium* (1.0×10^{10} cfu/mL)
Group C = Administered with β-glucans at dose rate of 50 mg/kg of feed + *Sal. typhimurium* challenge (1.0×10^{10} cfu/mL)
Group D = Administered with β-glucans at dose rate of 100 mg/kg of feed + *Sal. typhimurium* challenge (1.0×10^{10} cfu/mL)

The birds of all the groups were raised on the floor system under standard management conditions and provided with feed and water *ad libitum*. Prior approval was obtained from the Board of Studies of Department of Pathobiology followed by Advanced Studies and Research Board, Bahauddin Zakariya University, Pakistan (Edstt. No. Acad/MPhil/FVS/14-16/1178) regarding ethical concerns to conduct this experimental study.

Evaluation of immunotherapeutic effects against salmonellosis

Scoring of clinical signs and growth performance in experimental and control groups: Clinical signs and mortality were observed on a daily basis whereas growth performance was recorded in terms of feed conversion ratios (FCR) on weekly basis.

Histopathological studies: Intestinal tissues from birds of experimental and control groups were collected on days 13^{th} and 27^{th} post-challenge with *Sal. typhimurium* for histopathological examination by using standard protocol as described by Shao *et al*. (2013).

Relative weights of lymphoid organs: To assess the effect of β-glucans on the development of lymphoid organs which are involved in inducing an immune response, relative weights of lymphoid organs including bursa of Fabricius, spleen, and thymus were calculated using the methodology described by Awais *et al*. (2018).

Cell-mediated immune responses in experimental and control groups post-challenge with *Sal. typhimurium*: Mononuclear phagocytic system functioning in industrial broilers was determined by using the methodology described previously (Sarker *et al*., 2000). The basophilic hypersensitivity response was assessed by *in vivo* lymphoproliferative response to Phytohemagglutinin-P (PHA-P) following the method of Corrier (1990).

Serum biochemical profile: Alanine transferase (ALT), aspartate aminotransferase (AST), creatinine, cholesterol, triglycerides and glucose levels were determined using semi-automatic chemistry analyzer (BTS-350, Biosystems, Spain).

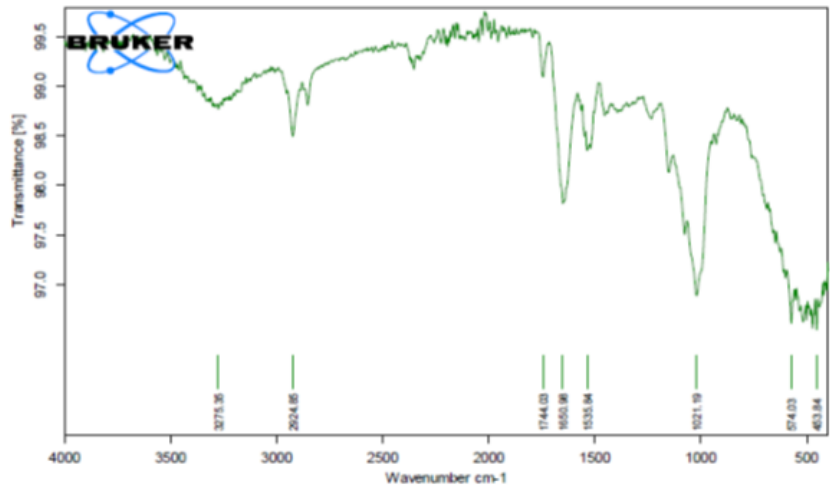

Fig. 1: Fourier Transform Infrared spectra of indigenous yeast-derived β-glucans using FTIR spectrometer

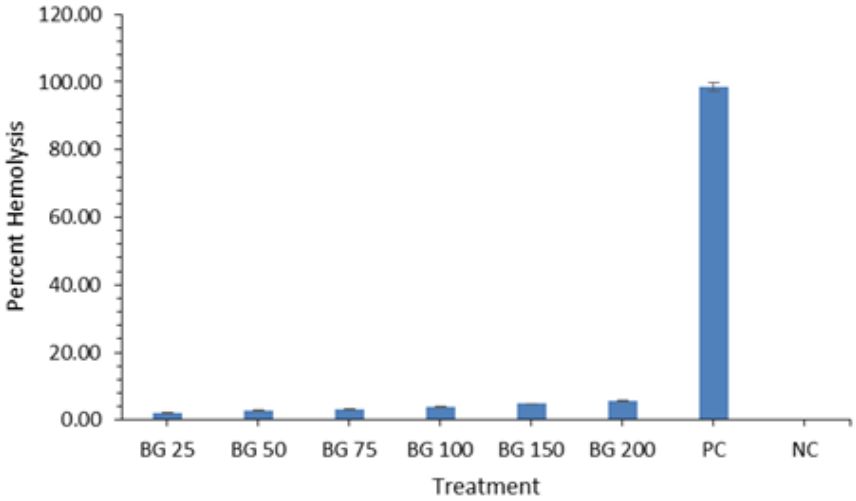

Fig. 2: Percent hemolysis of erythrocytes by yeast derived β-glucans (BG) at different concentrations, NC: Negative control; PC: Positive control

Statistical analysis: The data thus obtained were analyzed using one-way analysis of variance (ANOVA) and group means were compared by Duncan's multiple range (DMR) test using SPSS v25. The differences at $P<0.05$ were considered significant.

RESULTS

Identification and morphological characterization of *S. cerevisiae*: *S. cerevisiae* was isolated from baker's yeast, sugarcane sludge, molasses and identified based on the morphological characteristics including small, smooth creamy or white raised colonies on yeast peptone dextrose (YPD) agar plates. For confirmation of the yeast, budding stage of the isolates was observed under the microscope.

Purification and molecular characterization of yeast β-glucans: The yield of β-glucans after extraction was 36.67 ± 1.17% from yeast cells. The β-glucan extraction yield in the present study was very similar to the standard β-glucans (36.74 ± 1.13%) provided with the kit. The FT-IR analysis of isolated β-glucans showed its peaks at wave-length of 1021 cm^{-1} as shown in Fig. 1.

Hemolytic Activity: The hemolytic activity was assessed to check the safety of the β-glucans at various concentrations. Triton X-100 was used as a positive control, showing 100% hemolysis of RBCs, whereas phosphate buffered saline (PBS) was used as a negative control, showing no hemolysis. A concentration range of 5-200 µg/ml was selected for the detection of hemolytic activity because in previous studies β-glucans have been used at various safe concentrations in above mentioned range. Results revealed that all the concentrations showed less than 10% hemolysis and are considered to be safe for use as a supplement in animal feed. The percent hemolysis observed with different concentrations of β-glucans have been presented in Fig. 2.

Table 1: Mutagenic activity of β-glucans by using *Salmonella typhimurium* TA98 and TA100

Treatments	Positive wells/Total wells		Results	
	TA 98	TA100	TA 98	TA100
Background	33/96	49/96	-	-
*Standard	96/96	96/96	Toxic	Toxic
β-glucans (50 µg/ml)	0/96	0/96	Nm	Nm
β-glucans (100 µg/ml)	0/96	0/96	Nm	Nm
β-glucans (200 µg/ml)	0/96	0/96	Nm	Nm
β-glucans (300 µg/ml)	0/96	0/96	Nm	Nm
β-glucans (400 µg/ml)	0/96	0/96	Nm	Nm

*NaN_3 for TA100 and $K_2Cr_2O_7$ for TA 98; Nm = non-mutagenic

Mutagenic activity: The identification of chemicals or substances that might cause mutation(s) is vital in the safety evaluation process since mutagenic substances have the potential to induce cancer. For the demonstration of mutagenic activity of isolated β-glucans, Ames test was performed by using *Sal. typhimurium* TA100 and TA98. Yellow or turbid wells were assessed as positive in the mutagenic activity, whereas purple wells were classified as

Table 2: The lymphoid organs to body weight ratios (Mean±SD) at 1st and 2nd slaughtering

Groups	Thymus		Bursa		Spleen	
	1st slaughtering	2nd slaughtering	1st slaughtering	2nd slaughtering	1st slaughtering	2nd slaughtering
A	2.26±0.15^{c}	1.1±0.36^{a}	0.77±0.57^{ab}	1.13±0.65^{ab}	0.65±0.30^{a}	0.73±0.44^{a}
B	0.96±0.55^{a}	0.86±0.31^{a}	0.39±0.05^{a}	0.46±0.17^{a}	0.90±0.37^{ab}	0.84±0.39^{a}
C	1.83±0.14^{bc}	1.95±0.13^{b}	1.05±0.23^{b}	0.95±0.14^{bc}	1.23±0.11^{b}	0.91±0.17^{a}
D	1.3±0.30^{ab}	1.26±0.45^{a}	1.22±0.09^{b}	1.24±0.04^{c}	1.40±0.2^{b}	1.50±0.64^{a}

Values sharing similar letters in each column are statistically non-significant (P>0.05)

Table 3: Phagocytic Index as determined by Carbon clearance assay in chickens fed different dietary levels of yeast β-glucan (Mean±SD)

Groups	Response at 3 min	Response at 15 min
A	223.6±24.40^{a}	113.6±5.50^{a}
B	194.0±16.00^{a}	92.3±4.60^{b}
C	220.0±16.60^{a}	121.3±7.09^{bc}
D	223.3±16.60^{a}	126.3±9.00^{c}

Table 4: Lymphoproliferative response (mm; Mean±SD) to phytohemagglutinin-P in experimental and control groups

Groups	Response at 24 hours	Response at 48 hours	Response at 72 hours
A	1.34±0.05^{b}	1.14±0.05^{b}	0.83±0.11^{b}
B	0.6±0.2^{a}	0.67±0.03^{a}	0.2±0.09^{a}
C	1.4±0.1^{b}	1.2±0.1^{b}	0.78±0.10^{b}
D	1.38±0.07^{b}	1.07±0.06^{b}	0.83±0.05^{b}

Table 5: Serum biochemical profiles of chickens of experimental and control groups at 1st and 2nd slaughtering

Parameters	Slaughtering	Groups			
		A	B	C	D
Cholesterol	1st	2.84±0.05^{a}	4.4±0.50^{b}	3.03±0.13^{a}	3.03±0.12^{a}
(mg/dL)	2nd	3.4±0.65^{b}	15.57±0.13^{a}	3.18±0.3^{a}	3.20±0.23^{a}
Triglycerides	1st	3.93±0.47^{a}	5.68±0.39^{b}	4.46±0.3^{a}	4.49±0.52^{a}
(g/L)	2nd	4.32±0.06^{b}	5.56±0.5^{a}	4.06±0.14^{a}	3.78±0.4^{a}
Glucose	1st	254±11.3^{a}	482±3.3^{a}	269±13^{a}	283.3±18.4^{a}
(mg/dL)	2nd	247±27.6^{b}	358.6±29.0^{a}	241.3±13.3^{a}	254±25.0^{a}
Creatinine	1st	0.2±3.39^{a}	0.56±0.28^{b}	0.3±0.11^{ab}	0.36±0.05^{ab}
(mg/dL)	2nd	0.43±0.11^{b}	0.63±0.32^{a}	0.4±0.11^{a}	0.95±1.38^{a}
ALT (U/l)	1st	3.66±0.57^{a}	5.00±1.00^{a}	4.00±1.00^{a}	3.66±0.57^{a}
	2nd	3.3±0.50^{a}	4.3±0.50^{a}	4.0±0.00^{a}	4.0±0.00^{a}
AST (U/l)	1st	190±8.8^{a}	331±29.8^{b}	189.6±3.05^{a}	183±7.09^{a}
	2nd	138.6±15.0^{a}	187.3±53.6^{a}	150.6±28.3^{a}	162.2±16.6^{a}

negative. The number of positive wells must be considerably greater than the number of positive wells in the background plate for the substance to be mutagenic (spontaneous mutations). No yellow wells were detected in the blank plate however there were 33 yellow wells for TA98 and 49 yellow wells for TA 100 for the backdrop. The results of mutagenicity assay are presented in Table 1 showing that all tested concentrations of β-glucans were non-mutagenic.

Evaluation of immunotherapeutic effects of β-glucans clinical signs and growth performance post challenge with *Sal. typhimurium*: No clinical signs were seen in the birds from negative control group (group A) until the end of the experiment. While, in group B (positive control), the birds were depressed and less active with ruffled feathers. The birds of groups C and D administered with β-glucans showed better gut health signs as compared to group B. Similarly, the highest FCR was recorded in group B (2.75) indicating poor growth performance as compared to treatment groups (C and D). Low FCR value (1.9) in group D indicated improved growth performance in birds supplemented with 100 mg β-glucans /kg of feed.

Relative weight of lymphoid organs: The relative weights (lymphoid organ to body weight ratios) of the thymus, bursa and spleen at both slaughtering were comparatively higher in group C and D as compared to the control groups. However, for different organs, this difference was not statistically significant in a consistent manner after different slaughtering (Table 2).

Phagocytic activity by carbon clearance assay: The results of carbon clearance assay are presented in Table 3. After 3 minutes of injection, the phagocytic index of β-glucans administered groups (C and D) was statistically similar (P>0.05) as compared to both control groups (A and B). However, after 15 minutes, the phagocytic index was significantly higher (P≤0.05) in treatment groups as compared to the control groups.

Lymphoproliferative response to phytohemagglutinin (PHA-P): At 24 hours post-injection of PHA-P, the lymphoproliferative response was significantly higher (P≤0.05) in groups A, C and D as compared to positive control group B. However, the difference between β-glucans administered (C and D) and negative control (A) groups was statistically non-significant (P>0.05). A similar response was detected at 48 and 72 hours post-PHA-P injection.

Histopathological studies of intestine in *Sal. typhimurium* infected and healthy broilers: Normal intestinal villus height and crypt depth ratio was observed in group A (Fig. 3A). The *Sal. typhimurium* produces intestinal mucosal injury, as evidenced by the considerable decrease in villus height and crypt depth ratio in group B (Fig. 3B). However, group C and D showed a considerable improvement in villus height and crypt depth ratio (Fig. 3C and D).

Serum biochemical parameters of *Sal. typhimurium* infected and healthy broilers: Serum biochemical parameters of all the groups fed with different dietary levels of yeast β-glucans on the 20th and 34th days of age are presented in Table 5.

DISCUSSION

The colony morphology of isolated *S. cerevisiae* cells was similar as described by Kurtzman *et al*. (2011). Whereas, the extraction yield of β-glucans was 36.67 ± 1.17% from yeast cells that was higher than the observations of Liu *et al*. (2008) using similar method. The FTIR spectroscopy is a useful tool in monitoring structural changes in biopolymers (β-glucans) that determines their biological functions (Wang *et al*., 2008). In present study, the FT-IR spectra showed β-glucans (carbohydrates) peaks at wavelength of 1021.19 cm^{-1} which indicated higher molecular weight glucans with maximum β-type of linkages. This property makes them unique from other commercially available β-glucans regarding their biological

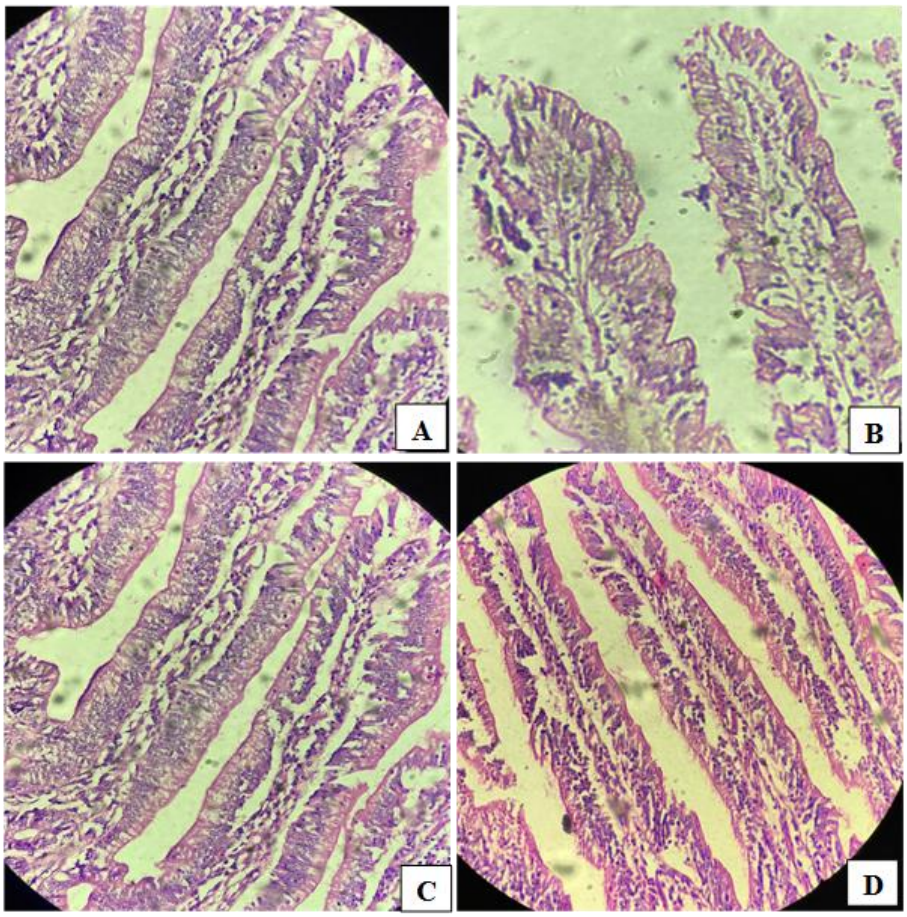

Fig. 3: Microphotograph of the small intestine (jejunum) showing (A) normal villi height and crypt depth ratio, (B) reduced density of villi, (C) dense villi with normal height and crypt depth and (D) improved villi height (40X, H&E staining).

functions. These findings are comparable with the results of Wang *et al*. (2008). These spectra also showed no absorbance at 840 cm^{-1} which revealed the absence of α-linked glycosidic bonds. Further, hemolytic activity was assessed to check the safety of the β-glucans at various concentrations i.e. 5-200 µg β-glucans/mL of PBS. Results revealed that all the concentrations showed less than 10% percent hemolysis and it is safe for use as a supplement in animal feed. Previously, Amin and Dannenfelser (2006) reported that compounds with a hemolysis value of <10% were considered non-hemolytic while those having values > 25% were considered to be at risk to cause hemolysis.

These yeast derived β-glucans were evaluated *in vivo* for their protective and immune modulating effects in broiler chicks challenged with *Sal. typhimurium*. The stock culture *Sal. typhimurium* was prepared in sterile PBS as described by Tarabees *et al*. (2017) and was adjusted to 1.0 of 10^{10} cfu/mL of PBS. In our study, significant differences were found in body weight gains and FCRs in groups administered with β-glucans. Previously, Zhang *et al*. (2008) reported that chickens reared in cages showed significant increase in body weight gains when supplemented with yeast β-glucans at the dose rates of 50 and 75 mg/kg of diet. Huff *et al*. (2006) reported improved body weight gains and feed consumption ratios in *Escherichia coli* challenged birds fed with 20 mg β-glucans/ton of feed. Literature also revealed that either in challenged or non-challenged broilers, supplantation of β-glucans do not adversely affect the performance of birds (Abd El-Wahab *et al*., 2019; Ashraf *et al*., 2019). It has also been demonstrated that β-glucans have a positive impact on intestinal morphology, increase goblet cell number and mucin-2 production in poultry, increase the flow of new immunocytes, enhance macrophage function, stimulate phagocytosis, induce increased expression of intestinal tight junctions, and function as potent anti-inflammatory immunomodulators (Schwartz and Vetvicka, 2021; Amer *et al*., 2022). Previously, Guo *et al*. (2003) reported that supplementation of yeast β-glucans raised the relative weights of the bursa and spleen. Cho *et al*. (2013) reported that 0.1% dietary supplementation of β-glucans resulted in improved relative weights of spleen and bursa of fabricius in unchallenged healthy birds. The addition of yeast β-glucans also improved the red blood cell count (Guo *et al*., 2003) and raised the villi height in jejunum (Morales-López *et al*., 2009) that increased the surface area for absorption of nutrients. Previous studies revealed that *Sal. typhimurium* invaded the liver and spleen and impaired the intestinal mucosal barrier. The birds which are challenged with *Sal. typhimurium* along with dietary treatment of yeast β-glucans showed improvement in intestinal health which is helpful in preventing the adhesion of invading of pathogens to the intestinal surface (Revolledo *et al*., 2009; Fasina *et al*., 2010). Some studies also proposed that the *Sal. typhimurium* increase the permeability of the intestinal barrier and thus facilitates the bacterial translocation (Köhler *et al*., 2007). Our study showed that the birds which were fed with yeast β-glucans showed a significant increase in villi height and crypt depth ratio, while the birds infected with *Sal. typhimurium* showed a decrease in the villi height and crypt depth ratio. Lymphoproliferative response to PHA-P showed lower response at 24 h level in basal diet group while both dietary treatment groups fed with yeast β-glucan maintained higher swelling response at

48 and 72 hours post PHA-P injection. Similar findings have been reported previously (Guo *et al*., 2003). The mononuclear phagocytic response was higher in the groups supplemented with β-glucans that corresponds to the protective efficacy of β-glucans. Khaliq *et al.* (2017) also reported similar findings in a study on immunotherapeutic effects of plant derived polysaccharides against *Eimeria* challenge in broilers.

Conclusions: Yeast β-glucan improved the gut health by reducing damage to intestinal mucosal barrier in broiler when challenged with *Sal. typhimurium.* These results provide new information on the role of yeast β-glucans in terms of immunity and growth performance in broilers challenged with bacterial population. Yeast β-glucans are the potential immunomodulators and can be successfully used as an alternative remedy to avoid the abundant use of antibiotics against bacterial pathogens including *Sal. typhimurium.*

Acknowledgements: This work was supported by Higher Education Commission (HEC), Govt. of Pakistan (vide grant number 7533) under National Research Program for Universities.

Authors contribution: MAT and AR performed the experiments, analyze the data and wrote the manuscript. MIA and MMA designed the experiments and supervised. FM, MA, MRH and NI helped in data analysis and interpretation. All authors review and approve the manuscript.

REFERENCES

Abd El-Wahab A, Mahmoud R, Marghani B, *et al.,* 2019. Effects of Yeast Addition to the Diet of Japanese Quails on Growth Performance, Selected Serum Parameters and Intestinal Morphology as well as Pathogens Reduction. Pak Vet J 40:219-23.

Abd Razak M-F, Aidoo KE and Candlish AG, 2007. Mutagenic and cytotoxic properties of three herbal plants from Southeast Asia. Trop Biomed 24:49-59.

Amer SA, Attia GA, Aljahmany AA, *et al.,* 2022. Effect of 1, 3-Beta Glucans Dietary Addition on the Growth, Intestinal Histology, Blood Biochemical Parameters, Immune Response, and Immune Expression of CD3 and CD20 in Broiler Chickens. Animals 12:3197.

Amin K and Dannenfelser RM, 2006. In vitro hemolysis: guidance for the pharmaceutical scientist. J Pharmaceu Sci 95:1173-76.

Ashraf S, Bhatti SA, Kamran Z, *et al.,* 2019. Assessment of Refined Functional Carbohydrates as Substitutes of Antibiotic Growth Promoters in Broilers: Effects on Growth Performance, Immune Responses, Intestinal Micro-Flora and Carcass Characteristics. Pak Vet J 39:157-62.

Awais MM, Akhtar M, Anwar MI, *et al.,* 2018. Evaluation of Saccharum officinarum L. bagasse-derived polysaccharides as native immunomodulatory and anticoccidial agents in broilers. Vet Parasitol 249:74-81.

Borchani C, Fonteyn F, Jamin G, *et al.,* 2016. Physical, functional and structural characterization of the cell wall fractions from baker's yeast Saccharomyces cerevisiae. Food Chem 194:1149-55.

Cho J, Zhang Z and Kim I, 2013. Effects of single or combined dietary supplementation of β-glucan and kefir on growth performance, blood characteristics and meat quality in broilers. Br Poult Sci 54:216-21.

Collett SR, Smith JA, Boulianne M, *et al.,* 2020. Principles of disease prevention, diagnosis, and control. Dis Poult 1-78.

Corrier DE, 1990. Comparison of phytohemagglutinin-induced cutaneous hypersensitivity reactions in the interdigital skin of broiler and layer chicks. Avian Dis 369-73.

Ding B, Zheng J, Wang X, *et al.,* 2019. Effects of dietary yeast beta-1, 3-1, 6-glucan on growth performance, intestinal morphology and chosen immunity parameters changes in Haidong chicks. Asian-australas J Anim Sci 32:1558.

Farrell D, 2013. The role of poultry in human nutrition. Poultry Development Review. Rome: Food and Agriculture Organization, 2-9.

Fasina Y, Hoerr F, McKee S, *et al.,* 2010. Influence of *Salmonella enterica* serovar Typhimurium infection on intestinal goblet cells and villous morphology in broiler chicks. Avian Dis 54:841-47.

Freimund S, Sauter M, Käppeli O, *et al.,* 2003. A new non-degrading isolation process for 1, 3-β-D-glucan of high purity from baker's yeast *Saccharomyces cerevisiae*. Carbohydr Polym 54:159-71.

Guo Y, Ali R and Qureshi M, 2003. The influence of β-glucan on immune responses in broiler chicks. Immunopharmacol Immunotoxicol 25:461-72.

Halwani M, Hossain Z, Khiyami MA, *et al.,* 2015. Liposomal β-glucan: preparation, characterization and anticancer activities. J Nanomed Nanotechnol 6:7.

Han B, Baruah K, Cox E, *et al.,* 2020. Structure-Functional Activity Relationship of β-Glucans From the Perspective of Immunomodulation: A Mini-Review. Front Immunol 11:658.

Hofmeyr I, 2019. Poultry nutrition, gut health, pathogens and the immune system: feed industry. AFMA Matrix 28:9-13.

Huff G, Huff W, Rath N and Tellez G, 2006. Limited treatment with β-1, 3/1, 6-glucan improves production values of broiler chickens challenged with *Escherichia coli*. Poult Sci 85:613-18.

Khaliq K, Akhtar M, Awais MM and Anwar MI, 2017. Evaluation of immunotherapeutic effects of *Aloe vera* polysaccharides against coccidiosis in chicken. Kafkas Univ Vet Fak Derg 23:895-901.

Kohler H, Sakaguchi T, Hurley BP, *et al.,* 2007. *Salmonella enterica* serovar Typhimurium regulates intercellular junction proteins and facilitates transepithelial neutrophil and bacterial passage. Am J Physiol Gastrointest Liver Physiol 293:G178-87.

Kurtzman CP, Fell JW and Boekhout T, 2011. Definition, classification and nomenclature of the yeasts, The yeasts, Elsevier, pp. 3-5.

Lei S, 2019. Yeast β-Glucans in Poultry Farming. J Immunol 13:45-9.

Liu X-Y, Wang Q, Cui SW, *et al.,* 2008. A new isolation method of β-D-glucans from spent yeast Saccharomyces cerevisiae. Food Hydrocoll 22:239-47.

Morales-López R, Auclair E, Garcia F, *et al*., 2009. Use of yeast cell walls; β-1, 3/1, 6-glucans; and mannoproteins in broiler chicken diets. Poult Sci 88:601-7.

Revolledo L, Ferreira C and Ferreira A, 2009. Prevention of *Salmonella Typhimurium* colonization and organ invasion by combination treatment in broiler chicks. Poult Sci 88:734-43.

Rezaei MN, Dornez E, Jacobs P, *et al.,* 2014. Harvesting yeast (*Saccharomyces cerevisiae*) at different physiological phases significantly affects its functionality in bread dough fermentation. Food Microbiol 39:108-115.

Sarker N, Tsudzuki M, Nishibori M, *et al.,* 2000. Cell-mediated and humoral immunity and phagocytic ability in chicken lines divergently selected for serum immunoglobulin M and G levels. Poultry Sci 79:1705-09.

Schwartz B and Vetvicka V, 2021. β-glucans as Effective Antibiotic Alternatives in Poultry. Molecul 26:3560.

Shao Y, Guo Y and Wang Z, 2013. β-1, 3/1, 6-Glucan alleviated intestinal mucosal barrier impairment of broiler chickens challenged with *Salmonella enterica* serovar Typhimurium. Poult Sci 92:1764-73.

Suresh G, Das RK, Kaur Brar S, *et al.,* 2018. Alternatives to antibiotics in poultry feed: molecular perspectives. Crit Rev Microbiol 44:318-35.

Tarabees R, Elsayed MS, Shawish R, *et al.,* 2017. Isolation and characterization of *Salmonella Enteritidis* and *Salmonella Typhimurium* from chicken meat in Egypt. J Infect Dev Ctries 11:314-9.

Wang Y, Ahmed Z, Feng W, *et al.,* 2008. Physicochemical properties of exopolysaccharide produced by *Lactobacillus kefiranofaciens* ZW3 isolated from Tibet kefir. Int J Biol Macromol 43:283-88.

Zechner-Krpan V, Petravić-Tominac V, Gospodarić I, *et al.,* 2010. Characterization of 13-Glucans Isolated from Brewer's Yeast and Dried by Different Methods. Food Technol Biotechnol 48:189-97.

Zhang B, Guo Y and Wang ZM, 2008. The Modulating Effect of 棺-1, 3/1, 6-glucan Supplementation in the Diet on Performance and Immunological Responses of Broiler Chickens. Asian-australas J Anim Sci 21:237-44.