p-ISSN 2615-787X e-ISSN 2615-790X
Accredited by Directorate General of Higher Education, Research, and Technology, Republic of Indonesia, No. 225/E/KPT/2022

Tropical Animal Science Journal, March 2024, 47(1):53-60
DOI: https://doi.org/10.5398/tasj.2024.47.1.53
Available online at https://journal.ipb.ac.id/index.php/tasj

Impact of ß-Glucan with Non-Glucan Biomaterials on Growth Performance, Carcass Characteristics, and Viable Count of Lactobacilli in Broiler Chicks

H. M. Hashaam[a], S. Naveed[a], S. Rehman[b], M. Zeeshan[c], A. Rahman[d,e], W. P. Lokapirnasari[f,*], M. A. Hussain[d], R. Begum[g], M. Jamal[h], & A. B. Yulianto[i]

[a]Department of Animal Nutrition, University of Veterinary and Animal Sciences, Lahore-54000, Pakistan
[b]Division of Veterinary Public Health, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia
[c]Department of Anatomy and Histology, University of Veterinary and Animal Sciences, Lahore-54000, Pakistan
[d]College of Veterinary Sciences and Animal Husbandry, Abdul Wali Khan University Mardan-23200, Pakistan
[e]Department of Medicine and Therapeutics, Faculty of Medicine, The Chinese University of Hong Kong
fDivision of Animal Husbandry, Faculty of Veterinary Medicine, Universitas Airlangga, Indonesia
[g]Department of Zoology, Timergara Campus, Abdul Wali Khan University Mardan, Pakistan
[h]Department of Microbiology, Abdul Wali Khan University Mardan, Pakistan
[i]Faculty of Veterinary Medicine, Universitas Wijaya Kusuma Surabaya, Indonesia
*Corresponding author: widya-p-l@fkh.unair.ac.id
(Received 30-06-2023; Revised 12-10-2023; Accepted 17-10-2023)

ABSTRACT

Probiotics, prebiotics, and immunomodulators like β-glucan have become popular feed additives. Thus, this study examined the effects of a β-glucan product fortified with dietary biomaterials (fats, proteins, and minerals) on broiler chicks' growth, carcass features, immunological response, white blood cell (WBC) count, and viable count (number of living cells) of lactobacilli. Day-old Ross-308 (n=250) were randomly assigned to 1 of 5 dietary treatments; A= basal diet, B= basal diet + 40 mg/kg of avilamycin, C= basal diet + 250 g/ton *β*-glucan product, D= basal diet + 500 g/ton β-glucan product, and E= basal diet + 750 g/ton β-glucan product. The starter diet was administered from days 1 to 14, the grower diet from days 15 to 21, and the finisher diet from days 22 to 35. Each treatment had 5 repetitions of 10 birds. On days 7 and 20, all birds were eye-drop inoculated against the Newcastle disease (ND) vaccine. Three chickens from each replication of all treatments were slaughtered on day 35 to examine carcass features and collect ileal digesta. White blood cell and viable lactobacilli counts at the end of the trial showed the effect of β-glucan supplementation. Throughout the trial, β-glucan administration did not increase average daily weight gain. The treatments did not change WBC or viable count; however, lactobacilli count increased (p≤0.05) in treatment group E. Treatment E increased (p≤0.05) ND-vaccination antibody-titers but did not affect immunological organ development. Treatment diet E (base diet +750 mg/t β-glucan product) improved broiler immunity and gut microbiota. In conclusion, the addition of β-glucan to broiler feed enhanced the beneficial gut flora, particularly *Lactobacilli* and immune response, and may serve as an alternative to antibiotics.

Keywords: ß-Glucan; antibody; broiler chicks; carcass; viable count

INTRODUCTION

Pakistan is the 11th largest poultry producer in the world, producing about 1.70 million tons of white meat annually, which is increasing yearly (Ali *et al.*, 2019). The challenging issue in broiler production units is formulating the least cost ration, which ensures 70% of total production cost (Maqsood *et al.*, 2022). The foremost contest for poultry industrialists is to acquire maximum performance while coping with the mortality in broilers. The inclusion of feed additives in poultry feed resolved this issue for nutritionists. These feed additives include antibiotic growth promoters (AGPs), probiotics, prebiotics, different enzymes, and coccidiostats. Supplementation of antibiotic growth promoters in poultry diets at a sub-therapeutic level has been a common practice for the last 60 years because of its optimization in growth performance, feed efficiency, and disease prevention (Castanon, 2007). The net effect of using antibiotics in poultry feed as growth promoters ensures 3%-5% better performance (Gadde *et al.*, 2017). The AGP benefits on broiler performance and reduction in pathogenic pressure are well documented. Supplementation of antibiotics at a sub-therapeutic level in poultry diet can precede an imbalance of microbiota in the early stages of life and thus it leads to compromised immunity in future, ultimately making chicks more susceptible to pathogenic microbes in later stages of life (Simon *et al.*, 2016). Some later researchers illustrated that regular use of AGPs in diet acquires antibiotic resistance in poultry (Cosby *et al.*, 2015). The indiscriminate use of AGPs in broilers resulted in

treatment failure, economic loss, and the development of antibiotic resistance and other adverse effects in humans (Darwish *et al.,* 2013).

There is a worldwide trend to decrease the use of antibiotics in poultry feed due to contamination of antibiotic residues in poultry products (Rayala *et al.,* 2017). Different types of supplements have been discovered to replace AGPs, which can also sustain performance and immunity (Biggs & Parsons, 2008). In this context, β-glucan products have been the most favorable feed additives possessing unique immunomodulation properties and can be used to replace AGPs. Beta-glucan is a homopolysaccharide constituent of the cell wall of fungi found in the form of 1-3 β-glucan with side branches of 1-6 β-glucan and a linear form is also found in unicellular alga *Euglena gracilis* (Bacic *et al.,* 2009). Beta-glucan has immunity-enhancing effects in animals as well as humans (Kil *et al.,* 2023). Beta-glucan is receiving attention as an immune modulator without compromising the production performance and has no undesirable effects on birds (Cox *et al.,* 2010). Linear forms of β-glucan have attained more interest from researchers for high production and bioavailability instead of branched ones because the primary innate immune receptors for 1-3 β-glucan are dectin-1 which are entirely expressed in monocytes (Haji *et al.,* 2022).

In monogastric diets, β-glucan has been of major interest as a potential prebiotic possessing immunomodulating properties (Zhou *et al.,* 2013; Tykałowski & Koncicki, 2022; Murphy *et al.,* 2013). Results suggested that the incorporation of 50% glucan and 50% non-glucan biomaterials (sugars, proteins, and fats) promoted the growth of both *Lactobacillus* and *Bifidobacterium* which are beneficial microorganisms and protect the gut environment from pathogenic microbes. Supplementation of β-glucan in the diet has beneficial effects by enhancing immune function in monogastric animals but has no adverse effects on growth performance (Horst *et al.,* 2019; Divya *et al.,* 2020; Zhou *et al.,* 2013).

The use of antibiotics as growth promoters in poultry feed has been banned in many countries due to concern over the emergence of antibiotic-resistant bacteria. This has led to the increased interest in alternative feed additives such as probiotics, prebiotics, and immunomodulators like β-glucan. Recently, there has been interest in combining β-glucan with non-glucan biomaterials such as chitosan, collagen, and alginate to enhance its efficacy as a feed additive. These biomaterials have been shown to have health benefits, including antibacterial and antioxidant properties, and may enhance the immune-modulating effects of β-glucan. Overall, using β-glucan as a feed additive for broilers shows promise as an alternative to antibiotics for improving growth performance and immune function. The combination of β-glucan with non-glucan biomaterials may further enhance its efficacy and provide additional health benefits. Therefore, the current study was conducted to fully understand the impact of β-glucan product on the broiler-chick's immune response, growth performance, carcass traits and gut microbial population.

MATERIALS & METHODS

Ethical Approval and Study Area

The current study was approved by the ethical committee of the Directorate of Advance Studies and Research Board (ASRB) of the University of Veterinary and Animal Sciences (UVAS) Lahore with ethical number DAS/918, and conducted at the poultry research and training center (PRTC), Ravi campus Pattoki. In the trial, 250 Ross 308 mixed-sexed were procured from the local hatchery. After flushing, chicks were checked for abnormalities.

Study Population and Experiment

All the chicks were weighed initially at day 0 and randomly divided into five treatment groups, each with five replicates using a completely randomized design (CRD). The total duration of the biological trial was 35 days, divided into starter, grower, and finisher phases, respectively. The starter diet was given from day 1 to 14, the grower diet was given from day 15 to 21, and the finisher diet was given from 22 days to 35 days. Aleta-a commercially available product of β-glucan fortified with dietary non-glucan biomaterial having a composition of 50% β-glucan and 50% non-glucan biomaterial (fat, protein, and minerals) was used in the experiment. Among the treatment groups, the 1st group was negative control with no antibiotics and no inclusion of β-glucan with non-glucan biomaterials in the diet, and the 2nd group was positive control with antibiotics and no inclusion of β-glucan with non-glucan biomaterials in the diet, the 3rd, 4th, and 5th treatment groups were allocated β-glucan with non-glucan biomaterials in the diet at the rate of 250 g/ton, 500 g/ton, and 750 g/ton, respectively. Both control groups were given a commercial corn-soya based diet. Chicks were given ad-libitum feed and water after arrival. All experimental rations were formulated according to the standard prescribed in the manual of Ross 308. The dietary ingredients and calculated nutrient contents of the diets required for the three fundamental phases of growth of broiler chicks are presented in Table 1. All management practices were followed during the trial.

The growth performance was measured in terms of feed intake, body weight gain, feed conversion ratio (FCR), and livability in terms of survival by international standard methods as mentioned in the manual of Ross 308. Carcass evaluation was done on the 35th day. Three birds per replicate were selected and subjected to pre-slaughter fasting for 10 hours. Later, birds were weighed to check the live weight at slaughter to determine carcass and carcass yield. Birds were slaughtered by the halal Muslim method (*Dhabihah*). Each bird was cut into different body parts according to the standard method for calculation of carcass weight, giblet weight (liver, heart, spleen, gizzard, and bursa), breast yield, and leg quarter yield (Silveira *et al.,* 2017). Antibody titers were checked for Newcastle disease (ND) vaccine using hemagglutination (HA) and hemagglutination inhibition (HI) tests.

Table 1. Dietary ingredients and nutrient contents (calculation) of the diets required for all the three different phases of broiler chicks (as-fed basis)

Items	Starter ration	Grower ration	Finisher ration
Ingredients (%)			
Maize	39.20	39.20	45.10
Wheat	10.00	10.00	10.00
Rice polish	7.70	7.70	8.00
Soy bean meal	16.40	16.40	17.30
Canola meal	19.0	19.0	11.40
Guar meal	3.00	3.00	3.00
Oil	2.50	2.50	2.50
Limestone	0.60	0.60	1.00
Bone ash	0.40	0.40	0.50
DL-meth	0.10	0.10	0.12
Lysine sulphate	0.40	0.40	0.32
Premix*	0.20	0.20	0.20
Choline	0.10	0.10	0.10
Soda bicarb	0.30	0.30	0.25
Salt	0.10	0.10	0.10
Zinc-bacitracin	0.01	0.01	0.01
L-threonine	0.01	0.01	0.04
Aleta	0.00	0.00	0.00
Total	100.00	100.00	100.00
Calculated composition			
ME (kcal/kg)	3150.00	3200.00	3250.00
DM (%)	92.30	92.40	94.50
CP %	22.70	22.20	21.20
Crude fiber (%)	4.43	6.30	5.21
Crude fat (%)	5.98	5.65	6.90
Ash (%)	7.70	7.88	7.71
Calcium (%)	1.50	0.95	0.80
Phosphorous (%)	0.50	0.45	0.39
Lysine (%)	1.20	1.00	0.85
Methionine (%)	0.50	0.40	0.52
Arginine (%	1.25	0.00	0.90
Cysteine (%)	0.00	1.10	0.00
Methionine + Cysteine (%)	0.90	0.74	8.40

Note: *Premix= Vitamin-mineral premix supplemented per ton of feed: Phytase 200, Manganese 110, Zinc 90, Niacin 50, Tocopherol 50, Iron 40, Copper 20, Pantothenate 18, Retinol 12, Riboflavin 9, Pyridoxine 5, Cholecalciferol 5, Menadione 3, Thiamine 3, Molybdenum 2, Folate 2, Iodine 1, Biotin 0.3, Selenium 0.3, Cobalt 0.25, Cobalamin added at rate of gram per ton of the feed.

Moreover, the immune response was also observed from the development of immune organs such as the spleen and bursa. The weights of the spleen and bursa were recorded from slaughtered birds of all treatment groups, and data were compared. Leukocytes were counted by using an automatic hematology analyzer. At slaughtering on day 35, samples of digesta were collected from 15 birds of each treatment through the proximal ileum into sterile plastic vials and stored at 4 °C temperature for 24 h until they were analyzed. One gram of sample was used and subjected to serial dilutions with sterile saline solution (0.9%) and was plated on deMan, Rogosa, Sharpe (MRS), and nutrient agar media. The plates were incubated at 37 °C for 24 hours. Bacterial colonies were computed in a colony counter expressed as $\log^{10}$ cfu/g of the sample (Bortoluzzi *et al.*, 2018). Gastrointestinal bacteria *Lactobacillus* was counted in the small intestine and digesta of the 15 selected birds per treatment on the 35^{th} day. Homogenized samples were diluted 10 folds with sterile Phosphate Buffer Saline (PBS). From each dilution, 1 µL was placed on a specific media plate of *Lactobacillus* and incubated aerobically at 37 °C for 24 hours. Visible colonies were computed in a colony counter and expressed as $\log^{10}$ cfu/g of the sample (Ghosh *et al.*, 2012).

Statistical Analysis

The data were analyzed by analysis of variance (one-way ANOVA) and expressed as the mean and standard error of the mean (SEM). The p-value<0.05 was considered statistically significant. The means of treatment groups were further challenged by Duncan's Multiple Range test (DMRT) for statistical differences.

RESULTS

Growth Performance

The dietary effects of β-glucan with non-glucan biomaterials treatment on the growth performance of the broiler over 35 days of the experimental period are presented in Table 2. The results indicated that feed intake was not affected by the supplementation of β-glucan compared with those fed with antibiotic and non-antibiotic diets. Feed intake was approximately the same during this period among the treatments, but numerically lower feed intake was observed in treatment A and higher in E. Body weight and FCR gain were not significantly influenced by the treatment of β-glucan in all experimental groups.

Carcass Traits

Data on carcass traits are presented in Table 3. The live weight of randomly selected birds was not improved significantly among all the treatment groups. Breast weight was higher in group D, and leg quarter weight was higher in group B among the treatments. There were no significant differences in the weight of the liver, gizzard, and heart among the dietary treatments.

Livability

The effects of β-glucan product on the livability percentage of broilers are summarized in Table 4. There was no significant difference observed in livability among all treatments during 35 days period. The numerical value of treatment E was 1.3% greater than A but not statistically significant.

Table 2. Growth performance of broiler chicks fed ration containing β-glucan and non-glucan biomaterials

Variables	Treatments	Age (days)					
		0-7	8-14	15-21	22-28	29-35	0-35
		(Starter)		(Grower)	(Finisher)		(Cumulative)
Feed intake	A	160.65	390.52	650.97	949.91	1093.09	3245.15
(g/bird/week)	B	154.05	387.18	663.97	942.46	1086.19	3233.87
	C	151.44	383.96	658.41	952.93	1092.51	3239.25
	D	158.44	387.16	652.04	949.55	1083.85	3231.02
	E	155.87	390.15	665.36	958.41	1084.26	3254.05
	Pooled SEM	1.99	2.55	4.44	5.52	6.07	9.05
	p -value	0.494	0.932	0.738	0.944	0.847	0.932
Body weight gain	A	135.96	296.25	483.39	563.76	582.17	2062.14
(g/bird/week)	B	147.46	310.90	476.75	574.48	596.46	2105.91
	C	137.61	319.36	464.54	569.50	600.38	2091.33
	D	144.87	307.29	480.26	601.76	631.05	2164.64
	E	141.61	298.92	471.88	586.04	605.92	2104.39
	Pooled SEM	1.91	5.11	5.11	5.81	8.18	15.30
	p-value	0.252	0.362	0.651	0.050	0.334	0.157
Feed conversion ratio	A	1.18	1.32	1.34	1.68	1.87	1.57
	B	1.04	1.24	1.39	1.64	1.82	1.53
	C	1.10	1.20	1.42	1.67	1.82	1.55
	D	1.09	1.26	1.36	1.58	1.72	1.49
	E	1.10	1.31	1.41	1.63	1.79	1.54
	Pooled SEM	0.01	0.02	0.01	0.02	0.02	0.01
	p-value	0.204	0.469	0.455	0.164	0.299	0.091

Note: A= basal diet, B= basal diet + 40 mg/kg of avilamycin, C= basal diet + 250 g/ton β-glucan product, D= basal diet + 500 g/ton β-glucan product, E= basal diet + 750 g/ton β-glucan product.

Table 3. Carcass traits of broiler chicks fed ration containing β-glucan with non-glucan biomaterials

Treatments	Variables							
	Live weight (g/bird)	Carcass weight (g/bird)	Carcass yield (%)	Breast weight (g/bird)	Leg quarter weight (g/bird)	Giblets weight (%)		
						Liver weight (%)	Gizzard weight (%)	Heart weight (%)
A	2163.60	1350.8	62.3	776.60	288.33	2.36	1.91	0.66
B	2205.06	1395.4	63.4	784.80	294.60	2.37	1.87	0.65
C	2196.73	1387.73	63.0	783.80	285.33	2.32	1.87	0.68
D	2247.73	1424.93	63.4	794.60	291.00	2.34	1.88	0.69
E	2244.86	1425.26	63.4	787.86	293.00	2.37	1.87	0.67
Pooled SEM	17.93	13.87	0.44	7.91	5.62	0.02	0.04	0.01
p-value	0.110	0.085	0.699	0.650	0.945	0.787	0.983	0.628

Note: A= basal diet, B= basal diet + 40 mg/kg of avilamycin, C= basal diet + 250 g/ton β-glucan product, D= basal diet + 500 g/ton β-glucan product, E= basal diet + 750 g/ton β-glucan product.

Table 4. Livability of broiler chicks fed ration containing β-glucan with non-glucan biomaterials (%)

Treatments	Observation time					Cumulative
	Week 1	Week 2	Week 3	Week 4	Week 5	
A	94	100	100	97.7	98	97.9
B	98	98	100	100	98	98
C	98	98	98	100	98	98.4
D	100	98	100	96	100	98.8
E	100	98	98	100	100	99.2
Pooled SEM	0.84	0.77	0.57	0.71	0.68	0.22
p-value	0.113	0.906	0.569	0.237	0.736	0.516

Note: A= basal diet, B= basal diet + 40 mg/kg of avilamycin, C= basal diet + 250 g/ton β-glucan product, D= basal diet + 500 g/ton β-glucan product, E= basal diet + 750 g/ton β-glucan product.

Table 5. White blood cell, viable count of Lactobacilli, and immune response in broiler chicks fed ration containing β-glucan with non-glucan biomaterials

Treatments	Variables					
	WBCs count $(10)^3$	Total viable count (log cfu)	*Lactobacilli* count (log cfu)	ND titer	Bursa weight (g)	Spleen weight (g)
A	24.13	6.89	5.26^{bc}	4.33^{bc}	1.80	2.85
B	23.80	6.73	5.01^{c}	4.13^{c}	1.73	2.94
C	24.73	6.88	5.38^{ab}	4.80^{abc}	1.79	2.88
D	25.00	6.89	5.74^{ab}	5.07^{ab}	1.76	2.99
E	25.20	6.95	5.91^{a}	5.33^{a}	1.77	3.09
Pooled SEM	0.43	0.04	0.11	0.19	0.07	0.06
p-value	0.4250	0.244	0.012	0.0180	0.9829	0.4113

Note: A= basal diet, B= basal diet + 40 mg/kg of avilamycin, C= basal diet + 250 g/ton β-glucan product, D= basal diet + 500 g/ton β-glucan product, E= basal diet + 750 g/ton β-glucan product. WBC= white blood cell; ND= Newcastle disease. Means in the same column with different superscripts differ significantly ($p<0.05$).

Leukocytes, Viable Count of Lactobacilli, and Immune Response

The blood profile, viable count, and immune response of birds fed different levels of β-glucan product are shown in Table 5. Dietary treatments of β-glucan products had no impact on total white blood cell count. Treatment E had a higher numerical value than treatments A and B but was not positively affected. Dietary treatments of the β-glucan product had no positive impact on the total viable count but significantly improved the intestinal beneficial *Lactobacilli* count among the treatments ($p<0.05$). *Lactobacilli* count in group E was significantly ($p<0.05$) higher than in group B; however, treatments A, C, and D remain unchanged. The antibody titer against NDV was influenced significantly ($p<0.05$) by the dietary supplementation of β-glucan product. The maximum titer was observed in treatment E. The weight of immune organs, including the spleen and bursa remains unaffected, while the numerical value of bursa was higher in treatment A and the weight of the spleen was elevated among treatment group E. Increasing the dietary level of β-glucan products had no effect on immune organ development among all the treatments.

DISCUSSION

It was hypothesized that β-glucan products may replace the antibiotics as growth promoters used in the feed of broilers without any adverse effect on overall bird performance to resolve the issue related to the development of antibiotic resistance in humans by the consumption of broiler meat and to overcome the gap of deficiency of animal origin protein.

Growth Performance

In the current study, no positive effect was observed on feed intake, BWG, and FCR among treatments, whereas higher numerical values were observed in broilers fed with commercially available β-glucan products. The current study's findings match the previous studies (Cox *et al.,* 2010; Tykałowski & Koncicki, 2022) in which supplementation of β-glucan had no effects on feed intake and body weight gain in broilers. Similar results had previously been reported (Holanda *et al.,* 2020; Maqsood *et al.,* 2022) that supplementation of β-glucan had no positive effects on average daily feed intake and gain-to-feed ratio (G:F ratio) in weanling monogastric animals which are also supported by the current findings. Compatible results with our study found (Niu *et al.,* 2022) that β-glucan extracted from Chinese herbs had no beneficial impact on growth performance. The results of the present study showed that dietary supplementation of β-glucan supported the birds in a similar way as that of antibiotics, which reduce the chances of disease outbreaks related to opportunistic pathogens, thereby allowing birds to perform optimally. Although the results from the negative control group are non-significant, higher numerical values are indicators of optimism in performance.

In contrast, some authors reported opposite the results with that of our study that supplementation of β-glucan improved growth performance in terms of body weight gain and FCR in some phases of growth in broilers (Cho *et al.,* 2013; Ding *et al.,* 2019; Tykałowski & Koncicki, 2022; Ahiwe *et al.,* 2021; Kiarie *et al.,* 2022). The reasons behind these contradicted results might be a difference in the breed of chicks, the difference in experimental design, challenging feed with *Escherichia coli,* and the immunomodulation potential of β-glucan. Some of these authors used different breeds of broilers like Haidong, Arbor Acres, and Cobb and also used different sources of origin of β-glucan (yeast derived β-glucan). There were also variations in the feed type they used during their experiments, such as challenging feed fortified with *E. coli.* The diversity in experimental conditions and design, like hypoxia-reared chicks and disease-challenged like coccidiosis-infected chicks under *β*-glucan treatment, might be other main factors responsible for this discrepancy.

Carcass Traits

Supplementation of β-glucan product has no improvement on carcass characteristics and weight of relative organs in broilers because β-glucan has been proven as an immune modulating agent which has no significant effects on breast weight and leg quarter

weight. The results of the current study are in line with the findings of the previous studies (Baurhoo *et al.,* 2009; Rahimi *et al.,* 2019; Kil *et al.,* 2023; Kiarie *et al.,* 2022) in which dietary supplementation of 1-3 and 1-6 β-glucan did not improve the carcass yield, dressing percentage, relative carcass weight, and weight of visceral organs.

Livability

Livability percentage is an index of broiler production which has been improved by different satisfactory methods. In the current study, broiler chicks supplemented with β-glucan product have ameliorated survival rate as compared to control groups by augmenting the immune system for pathogenic protection or pathogenic susceptibility. The results of our study are compatible with the observations found in previous studies (Tykałowski & Koncicki, 2022; Moon *et al.,* 2016; Ngunyangi *et al.,* 2019). In a single previous study, contrasting outcomes may be due to heat stress, different precursors of prebiotics, and inclusion levels in the diet of broilers (Kil *et al.,* 2023).

Leukocytes, Viable Count of Lactobacilli, and Immune Response

The β-glucan attracted poultry researchers in recent years because of its immune-modulating potential by enhancing the proliferation of white blood cells. However, the absence of a challenge in the trial did not affect the WBC, lymphocyte, or IgG levels among treatments (Ngunyangi *et al.,* 2019; Zhang *et al.,* 2012), justifying the results of our study that supplementation of β-glucan does not affect WBC count as compared to the control group. In opposite to our findings, beneficial effects of β-glucan have been reported on adaptive immunity and proliferation of macrophages with increased phagocytosis (Amer *et al.,* 2022) that significantly improved the white blood cells and lymphocytes proliferation in chicks and mice respectively (Ding *et al.,* 2019), this may be due to the bioactivator properties of β-glucan.

Intestinal microbial populations directly negatively or positively affect an organism's performance. β-glucan, a non-starch polysaccharide, had been used as a prebiotic for encouraging the population of beneficial bacteria. In the current study, results showed a positive increase in numbers of *Lactobacilli* with β-glucan supplementation and supporting the findings of the previous studies (Goh *et al.,* 2023) which reported that β-glucan supplementation increased beneficial bacteria in monogastric animals. Similar results have also been reported that barley extracted β-glucan enhanced health-friendly microbes in older healthy human volunteers (Song *et al.,* 2021). The outcomes of our study are in line with the previous findings (Allen & Stabler, 2008), in which it was claimed that prebiotics contribute to the proliferation of beneficial bacteria and improve the microbial diversity in the gut. The concentration of β-glucan as a prebiotic affects the intestinal microbial community and selectively favored the butyrate-producing bacteria. The latest research studies have proved that prebiotics are more likely to be used by bacteria such as *Lactobacillus* to produce large amounts of short-chain fatty acids which rapidly reduce the gut pH. This manipulation suppresses the growth of pH-sensitive *E. coli* (Bader *et al.,* 2019). In coincidence with our hypothesis, the beneficial gut microbes halt the colonization of pathogenic bacteria and are also recognized for exporting bacteriocins, which can target and kill invading pathogens. Another piece of evidence proved that *E. coli* colonization is replaced with *Lactobacilli* in the gut by supplementing of prebiotics (Okrouhlá *et al.,* 2020). The significant decline in the growth of *E. coli* and coliform (Nakashima *et al.,* 2018) due to extended colonization of *Lactobacilli* is beneficial for optimum performance chicken, supporting our hypothesis.

Being relevant to our study, β-glucan has been proven as an immunity-enhancing agent and augmented protective mechanism against infectious diseases (Nakashima *et al.,* 2018). Due to the immune-activating response of β-glucan, many studies have been conducted in an infectious challenging condition and found that β-glucan has a magical response in activating the immune system (Tykałowski & Koncicki, 2022). The results of our study match the previous findings that β-glucan is a magnifying agent for plasma proteins IgG and IgA in broilers, which contribute to up-regulation of humoral immune response (Zhang *et al.,* 2012). Dietary β-glucan has been proven to increase the size of the primary and secondary lymphoid organs, providing further evidence of their immunomodulation capabilities (Amer *et al.,* 2022). Similar supporting observations were reported in other previous studies that supplementation of β-glucan showed significantly higher serum antibodies and high relative weight of bursa of Fabricius as compared to the non-treated group.

In a few previous studies, inconsistent results were reported that supplementation of β-glucan has no significant effects on the size of primary and secondary lymphoid organs, including bursa and spleen in broilers (Haghshenas *et al.,* 2014). Similar contradicted results were also reported that there is no significant impact of β-glucan supplementation in diet on ND vaccine titer in broilers. This may be due to the difference in sex, age group, and experimental conditions (Haghshenas *et al.,* 2014). The differences in experimental conditions and design might be the main factors responsible for the disparity of some of our findings.

CONCLUSION

The overall findings of the current study indicated that feeding commercially available β-glucan product (Aleta) enhanced gut-beneficial microbes, such as *Lactobacilli.* The supplementation of the product further enhanced the immune response of vaccines against ND in broilers. Hence, it is concluded that the use of β-glucan might benefit the health status of the chicken but not the production parameters as well as be safe for human health, having the potential to replace the prophylactic antibiotics application in the feed without compromising the production performance of broilers.

CONFLICT OF INTEREST

The authors have declared no hidden potential conflict of interest.

ACKNOWLEDGEMENT

The authors are highly indebted for the kind support provided by the Department of Animal Nutrition UVAS Lahore, Pakistan, for the smooth conduction of the current study.

REFERENCES

Ali, Z., I. Hussain, M. Faisal, A. M. Shoukry, S. Gani, & I. Ahmad. 2019. A framework to identify homogeneous drought characterization regions. Theor. Appl. Climatol. 137:3161-3172. https://doi.org/10.1007/s00704-019-02797-w

Allen, R. H. & S. P. Stabler. 2008. Identification and quantitation of cobalamin and cobalamin analogues in human feces. Am. J. Clin. Nutr. 87:1324–1335. https://doi.org/10.1093/ajcn/87.5.1324

Ahiwe, E. U., T. T. Dos Santos, H. Graham, & P. A. Iji. 2021. Can probiotic or prebiotic yeast (*Saccharomyces cerevisiae*) serve as alternatives to in-feed antibiotics for healthy or disease-challenged broiler chickens?: a review. J. Appl. Poult. Res. 30:100164. https://doi.org/10.1016/j.japr.2021.100164

Amer, S. A., G. A. Attia, A. A. Aljahmany, A. K. Mohamed, A. A. Ali, A. Goud, G. N. Alagmy, H. M. Megahed, T. Saber, & M. Farahat. 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. https://doi.org/10.3390/ani12223197

Bacic, A., G. B. Fincher, & B. A Stone. 2009. Chemistry, Biochemistry, and Biology of 1-3 Beta Glucans and Related Polysaccharides. 1st Edition, Academic Press, Elsevier. https://doi.org/10.1016/B978-0-12-373971-1.X0001-5

Bader, S., S. Gerbig, B. Spengler, A. Schwiertz, G. Breves, & M. Diener. 2019. Robustness of the non-neuronal cholinergic system in rat large intestine against luminal challenges. Pflugers Arch. 471:605–618. https://doi.org/10.1007/s00424-018-2236-7

Baurhoo, B., P. R. Ferket, & X. Zhao. 2009. Effects of diets containing different concentrations of mannanoligosaccharide or antibiotics on growth performance, intestinal development, cecal and litter microbial populations, and carcass parameters of broilers. Poult. Sci. 88:2262–2272. https://doi.org/10.3382/ps.2008-00562

Biggs, P. & C. M. Parsons. 2008. The effects of several organic acids on growth performance, nutrient digestibilities, and cecal microbial populations in young chicks. Poult. Sci. 87:2581–2589. https://doi.org/10.3382/ps.2008-00080

Bortoluzzi, C., J. G. M. Barbosa, R. Pereira, N. S. Fagundes, J. M. Rafael, & J. F. M. Menten. 2018. Autolyzed yeast (*Saccharomyces cerevisiae*) supplementation improves performance while modulating the intestinal immune-system and microbiology of broiler chickens. Front. Sustain. Food Syst. 2:85. https://doi.org/10.3389/fsufs.2018.00085

Castanon, J. I. 2007. History of the use of antibiotic as growth promoters in European poultry feeds. Poult. Sci. 86:2466–2471. https://doi.org/10.3382/ps.2007-00249

Cho, J. H., Z. F. Zhang, & I. H. Kim. 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. 54:216–221. https://doi.org/10.1080/00071668.2013.777691

Cosby, D. E., N. A. Cox, M. A. Harrison, J. L. Wilson, R. J. Buhr, & P. J. Fedorka-Cray. 2015. *Salmonella* and antimicrobial resistance in broilers: A review. J. Appl. Poult. Res. 24:408-426. https://doi.org/10.3382/japr/pfv038

Cox, C. M., L. H. Sumners, S. Kim, A. P. McElroy, M. R. Bedford, & R. A. Dalloul. 2010. Immune responses to dietary beta-glucan in broiler chicks during an Eimeria challenge. Poult. Sci. 89:2597–2607. https://doi.org/10.3382/ps.2010-00987

Darwish, W. S., E. A. Eldaly, M. T. El-Abbasy, Y. Ikenaka, S. Nakayama, & M. Ishizuka. 2013. Antibiotic residues in food: The African scenario. Jpn. J. Vet. Res. 61:S13–S22.

Ding, B., J. Zheng, X. Wang, L. Zhang, D. Sun, Q. Xing, A. Pirone, & B. Fronte. 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. 32:1558–1564. https://doi.org/10.5713/ajas.18.0962

Divya, M., N. Gopi, A. Iswarya, M. Govindarajan, N. S. Alharbi, S. Kadaikunnan, J. M. Khaled, T. N. Almanaa, & B. Vaseeharan. 2020. β-glucan extracted from eukaryotic single-celled microorganism *Saccharomyces cerevisiae*: Dietary supplementation and enhanced ammonia stress tolerance on *Oreochromis mossambicus*. Microbial Pathogenesis 139:103917. https://doi.org/10.1016/j.micpath.2019.103917

Gadde, U., W. H. Kim, S. T. Oh, & H. S. Lillehoj. 2017. Alternatives to antibiotics for maximizing growth performance and feed efficiency in poultry: A review. Animal Health Research Reviews 18:26-45. https://doi.org/10.1017/S1466252316000207

Ghosh, T. K., S. Haldar, M. R. Bedford, N. Muthusami, & I. Samanta. 2012. Assessment of yeast cell wall as replacements for antibiotic growth promoters in broiler diets: Effects on performance, intestinal histo-morphology and humoral immune responses. J. Anim. Physiol. Anim. Nutr. 96:275–284. https://doi.org/10.1111/j.1439-0396.2011.01155.x

Goh, T. W., H. J. Kim, K. Moon, C. S. Kim, & Y. Y. Kim. 2023. Effects of β-glucan with vitamin E supplementation on the growth performance, blood profiles, immune response, fecal microbiota, fecal score, and nutrient digestibility in weaning pigs. Anim. Biosci. 36:642–653. https://doi.org/10.5713/ab.22.0311

Haghshenas, M., H. Hosseini, K. Nayebzadeh, A. M. Khanghah, B. S. Kakesh, & R. K. Fonood. 2014. Production of prebiotic functional shrimp nuggets using ß-glucan and reduction of oil absorption by carboxymethyl cellulose: Impacts on sensory and physical properties. Journal Aquaculture Research Development 5:245-248. https://doi.org/10.4172/2155-9546.1000245

Haji, S., T. Ito, C. Guenther, M. Nakano, T. Shimizu, D. Mori, Y. Chiba, M. Tanaka, S. K. Mishra, J. A. Willment, G. D. Brown, M. Nagae, & S. Yamasaki. 2022. Human Dectin-1 is *O*-glycosylated and serves as a ligand for C-type lectin receptor CLEC-2. eLife 11:e83037. https://doi.org/10.7554/eLife.83037

Holanda, D. M., A. Yiannikouris, & S. W. Kim. 2020. Investigation of the efficacy of a postbiotic yeast cell wall-based blend on newly-weaned pigs under a dietary challenge of multiple mycotoxins with emphasis on deoxynivalenol. Toxins 12:504. https://doi.org/10.3390/toxins12080504

Horst, G., R. Levine, R. Chick, & C. Hofacre. 2019. Effects of beta-1,3-glucan (AletaTM) on vaccination response in broiler chickens. Poult. Sci. 98:1643–1647. https://doi.org/10.3382/ps/pey523

Kiarie, E. G., M. Mohammadigheisar, & H. Schulze. 2022. Effects of early feeding of enzymatically treated yeast on growth performance, organ weights, intestinal

histomorphology, and ceca microbial metabolites in broiler chickens subjected to Eimeria challenge. Poult. Sci. 101:101967. https://doi.org/10.1016/j.psj.2022.101967

Kil, B. J., Y. J. Pyung, H. Park, J. W. Kang, C. H. Yun, & C. S. Huh. 2023. Probiotic potential of *Saccharomyces cerevisiae* GILA with alleviating intestinal inflammation in a dextran sulfate sodium induced colitis mouse model. Sci. Rep. 13:6687. https://doi.org/10.1038/s41598-023-33958-7

Maqsood, M. A., E. U. Khan, S. N. Qaisrani, M. A. Rashid, M. S. Shaheen, A. Nazir, H. Talib, & S. Ahmad. 2022. Interactive effect of amino acids balanced at ideal lysine ratio and exogenous protease supplemented to low CP diet on growth performance, carcass traits, gut morphology, and serum metabolites in broiler chicken. Trop. Anim. Health Prod. 54:186. https://doi.org/10.1007/s11250-022-03184-w

Moon, S. H., I. Lee, X. Feng, H. Y. Lee, J. Kim, & D. U. Ahn. 2016. Effect of dietary beta-glucan on the performance of broilers and the quality of broiler breast Meat. Asian-Australas. J. Anim. Sci. 29:384–389. https://doi.org/10.5713/ajas.15.0141

Murphy, P., F. D. Bello, J. O'Doherty, E. K. Arendt, T. Sweeney, & A. Coffey. 2013. Analysis of bacterial community shifts in the gastrointestinal tract of pigs fed diets supplemented with β-glucan from *Laminaria digitata*, *Laminaria hyperborea* and *Saccharomyces cerevisiae*. Animal 7:1079–1087. https://doi.org/10.1017/S1751731113000165

Nakashima, A., K. Yamada, O. Iwata, R. Sugimoto, K. Atsuji, T. Ogawa, N. Ishibashi-Ohgo, & K. Suzuki. 2018. β-Glucan in foods and its physiological functions. J. Nutr. Sci. Vitaminol 64:8–17. https://doi.org/10.3177/jnsv.64.8

Ngunyangi, E. N., J. K. Tuitoek, M. K. Ambula, & A. M. Wachira. 2019. Evaluation of the antibiotic properties of probiotics and their efficacy on performance and immune response in broiler chicken. Int. J. Poult. Sci. 18:393-403. https://doi.org/10.3923/ijps.2019.393.403

Niu, X., Y. Ding, S. Chen, R. Gooneratne, & X. Ju. 2022. Effect of immune stress on growth performance and immune functions of livestock: mechanisms and prevention. Animals 12:909. https://doi.org/10.3390/ani12070909

Okrouhlá, M., J. Čítek, R. Švejstil, K. Zadinová, K. Pokorná, D. Urbanová, & R. Stupka. 2020. The effect of dietary *Helianthus tuberosus* L. on the populations of pig faecal bacteria and the prevalence of skatole. Animals 10:693. https://doi.org/10.3390/ani10040693

Rahimi, S., S. Kathariou, O. Fletcher, & J. L. Grimes. 2019. Effect of a direct-fed microbial and prebiotic on performance and intestinal histomorophology of turkey poults challenged with *Salmonella* and *Campylobacter*. Poult. Sci. 98:6572–6578. https://doi.org/10.3382/ps/pez436

Rayala R. V., V. R. Reddy, A. K. Panda, V.C. Preetam, & B. P. Reddy. 2017. Effect of dietary supplementation of antibiotic growth promoters vs synbiotics on growth performance, carcass characteristics and immune response of broiler chickens. Int. J. Curr. Res. 9:49718-49723.

Silveira, M. M., J. M. S. Martins, F. Litz, C. M. C. Carvalho, C. A. Moraes, M. C. A. Silva, & E. A. Fernandes. 2017. Effect of sorghum based nutritional programs on performance, carcass yield and composition of breast in broilers. Rev. Bras. Cienc. Avic. 19:43-50. https://doi.org/10.1590/1806-9061-2016-0253

Simon, K., M. B. Verwoolde, J. Zhang, H. Smidt, G. de V. Reilingh, B. Kemp, & A. Lammers. 2016. Long-term effects of early life microbiota disturbance on adaptive immunity in laying hens. Poult. Sci. 95:1543–1554. https://doi.org/10.3382/ps/pew088

Song, Q., Y. Wang, L. Huang, M. Shen, Y. Yu, Q. Yu, Y. Chen, & J. Xie. 2021. Review of the relationships among polysaccharides, gut microbiota, and human health. Food Res. Int. 140:109858. https://doi.org/10.1016/j.foodres.2020.109858

Tykałowski, B. & A. Koncicki. 2022. Effect of immunomodulation in Turkeys infected with haemorrhagic enteritis virus on the percentage of $CD4^+$ and $CD8\alpha^+$ T lymphocyte subpopulations synthesising IFN-γ. J. Vet. Res. 66:537–547. https://doi.org/10.2478/jvetres-2022-0068

Zhang, Z. F., T. X. Zhou, X. Ao, & I. H. Kim. 2012. Effects of β-glucan and Bacillus subtilis on growth performance, blood profiles, relative organ weight and meat quality in broilers fed maize–soybean meal-based diets. Livest. Sci. 150:419-424. https://doi.org/10.1016/j.livsci.2012.10.003

Zhou, T. X., J. H. Jung, Z. F. Zhang, & I. H. Kim. 2013. Effect of dietary β-glucan on growth performance, fecal microbial shedding and immunological responses after lipopolysaccharide challenge in weaned pigs. Anim. Feed Sci. Technol. 179:85-92. https://doi.org/10.1016/j.anifeedsci.2012.10.008