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Evaluation of a yeast-derived β-1,3/1,6-glucan preparation as a potential immunopotentiator for live La Sota Newcastle disease vaccination in broiler chickens

Wesam Alqwidi [1], Mohamed A. Marzok [1]*, Mahmoud Mohamed [1], Ibrahim El-Sabagh [2], Mohamed El-Sherif [3]*, Mohammed Abdel Hafez [4], Mohamed Mohamed [4], Mohamed Soliman [5]

[1] *Department of Clinical Studies, College of Veterinary Medicine, King Faisal University, al-Ahsa 31982, Saudi Arabia*
[2] *Central Biotechnology Laboratory, College of Veterinary Medicine, King Faisal University, al-Ahsa 31982, Saudi Arabia*
[3] *Department of Clinical Sciences, Faculty of Veterinary Medicine New Valley University, Al-Kharga 27511, Egypt*
[4] *Department of Avian and Rabbit Medicine, Faculty of Veterinary Medicine New Valley University, Al-Kharga 27511, Egypt*
[5] *Department of Poultry and Fish Diseases, Faculty of Veterinary Medicine Elminia University, Minia 61519, Egypt*

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* *Corresponding authors:*
Mohamed A. Marzok
Email: mmarzok@kfu.edu.sa
Mohamed El-Sherif
Email: mohamedelsherif@vet.nvu.edu.eg

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Abstract

Newcastle disease (ND) remains a constraint in vaccinated broiler chicken flocks, and practical immunopotentiators compatible with mass administration warrant evaluation. This exploratory study assessed short courses of a yeast-derived, water-dispersible preparation containing approximately 80% β-1,3/1,6-glucan in broilers receiving a single live La Sota vaccination. Three hundred day-old male Ross 308 chicks were allocated to five groups of 60 birds with four replicate pens per group: unvaccinated control (Control), vaccinated only (Vac), and vaccinated plus glucan at 0.5, 1, or 2 g/L in the drinking water (Vac β-Low, Vac β-Med, and Vac β-High, respectively). Glucan was administered on days 6–10, 21–22, and 28–29; the vaccine was administered by eye drop on day 7. Body weight, relative spleen, bursa, and thymus indices, hemagglutination-inhibition (HI) antibody titers; and 7-day survival following challenge on day 35 were recorded. The growth records showed an implausibly large increase between days 21 and 28 and could not be independently verified from the available data; consequently, the growth findings were presented as descriptive, and no claim of a growth effect or absence of a growth penalty was made. Exploratory organ index comparisons indicated that compared to Vac, Vac β-Med and Vac β-High had higher spleen indices on day 14, whereas only Vac β-High differed significantly from Vac on day 28. The age-wise analysis revealed that day-21 HI titer was higher in the Vac β-Med group than in the Vac group; whereas day-28 titers did not differ significantly. The day 42 samples were obtained from survivors only and therefore cannot be interpreted as an uncomplicated vaccine-response endpoint. The survival rates were 20%, 60%, 70%, 90%, and 80% in the control, Vac, Vac β-Low, Vac β-Med, and Vac β-High groups, respectively. The difference in the survival between Vac β-Med and Vac groups was 30 percentage points (approximate 95% CI: 2 to 52), but the exact odds ratio was imprecise (5.7, 95% CI: 0.9–64.6), and the comparison was not significant after the Holm adjustment (adjusted p = 0.19). Because each treatment was represented by a single challenge unit, bird-level survival comparisons are potentially pseudoreplicated. This study provides an exploratory signal for further testing but does not establish protection, an optimal dose, or an independent immunostimulatory mechanism.

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1. Introduction

Newcastle disease (ND), caused by virulent strains of avian orthoavulavirus 1 (Newcastle disease virus, NDV), remains among the major constraints to commercial poultry production worldwide (Dimitrov et al. 2017). Virulent viruses of genotype VII circulate endemically in the Middle East and North Africa, and outbreaks continue to occur in flocks vaccinated under intensive programs (Miller et al. 2010; Dimitrov et al. 2019; Sultan et al.al 2020; Lebdah al. 2024). Live lentogenic vaccines such as La Sota are the backbone of broiler programs because they are inexpensive and can be applied to whole flocks by eye drop, spray or drinking water; however, the immune response to a single early vaccination is variable. Maternally derived antibodies (MAs) can neutralize vaccine viruses and blunt the primary response in young chicks (Vrdoljak et al. 2017), genotype mismatch between vaccine and field viruses may reduce protection against viral shedding (Sultan et al. 2020), and vaccines that protect against clinical disease may not prevent shedding after challenge with highly virulent viruses (Kapczynski and King 2005). Practical measures that boost the immune response without changing vaccine manufacture or application are therefore of interest. Adjuvants and immunostimulants, particularly based on plant and microbial polysaccharides, have been shown to increase NDV antibody titers and T-cell responses in chickens (Aguilar and Rodríguez 2007; Wang et al. 2013; Zhao et al. 2016; Wu et al. 2022).

β-Glucans are glucose polymers whose biological activity depends on their source, linkage pattern (β-1,3 with or without β-1,6 branches), molecular mass and physical state (Goodridge et al. 2009). β-1,3-glucans are recognized by Dectin-1 and complement receptor 3 on macrophages, neutrophils and dendritic cells (Brown and Gordon 2001; Goodridge et al. 2009), and exposure to β-glucan can reprogram innate cells toward increased responses to later stimuli, a process termed trained immunity (Quintin et al. 2012; Netea et al. 2016; Walachowski et al. 2017). These properties suggest that β-glucans may improve the immunogenicity of live vaccines, although receptor usage and signaling in chickens are less well defined than they are in mammals. In poultry, β-glucan products increase antibody responses to avian influenza vaccines (Le et al. 2011) and alter vaccination responses in broilers (Horst et al. 2019), and several studies have addressed ND specifically. β-glucan combined with mannan-oligosaccharide increased HI titers and improved protection after virulent challenge in Egyptian broilers (Tamam et al. 2010); dietary mushroom β-glucan conferred dose-dependent protection against virulent NDV (Muthusamy et al. 2020),

and a yeast cell wall product enhanced cytokine production and lymphocyte proliferation in NDV-vaccinated broilers (Bi et al. 2022). However, the reported results are not consistent across studies, as the dietary β-glucan product increased ND titers only at the highest inclusion level, with growth and immune organ development remaining unchanged (Hashaam et al. 2024).

Most of the above studies used crude or composite products (for example, yeast cell walls or glucan–mannan combinations), continuous in-feed supplementation or a limited set of outcome parameters. To our knowledge, only a few studies have examined short-term delivery of a β-1,3/1,6-glucan in the drinking water around the time of vaccination while simultaneously characterizing the dose–response across lymphoid organ indices, antibody kinetics and survival after virulent challenge. Short water-delivered courses are attractive because they can be given on the day of vaccination without reformulating feed and may limit cost and chronic immune stimulation. The present study therefore evaluated whether short courses of a drinking-water preparation containing approximately 80% β-1,3/1,6-glucan, administered around a single live La Sota vaccination, were associated with changes in selected exploratory immunological endpoints and survival after virulent NDV challenge. The three concentrations (0.5, 1, and 2 g/L) were examined as a dose-ranging series. Because the product was administered separately from the vaccine antigen, the term "potential immunopotentiator" is used rather than a formulated adjuvant. The design estimates the effect of adding this regimen to the vaccination; without a glucan-only group, independent immunostimulatory activity cannot be established.

2. Materials and Methods

2.1 Ethics and reporting

All procedures were approved by the Institutional Animal Care and Use Committee of King Faisal University, with approval number KFU266679/2026. The study was conducted and reported according to the ARRIVE 2.0 guidelines (Percie du Sert et al. 2020).

2.2 Birds and husbandry

A total of 300 day-old male Ross 307 broiler chicken were procured from a commercial hatchery. The birds were not specific-pathogen-free, and their maternal NDV antibody titers and *Mycoplasma gallisepticum* status were not determined at the time of placement. The chicks were housed in battery brooders at 34 °C with continuous lighting for 3 days of acclimatization. Thereafter, there was a gradual reduction of temperature and photoperiod to standard commercial conditions. Commercial broiler rations (a nonmedicated commercial starter–grower–finisher broiler ration formulated to meet Ross 308 nutrient recommendations) and water were available *ad libitum*.

2.3 Test substance

β-1,3/1,6-Glucan was supplied as a white powder by the PVADO Company for Veterinary Medicines (Cairo, Egypt). The product was a yeast-derived, water-dispersible β-glucan preparation containing approximately 80% β-1,3/1,6-glucan. However, the degree of branching, particle-size distribution, and endotoxin content were not reported by the supplier. The powder was dissolved or dispersed in drinking water at 0.5, 1, or 2 g/L. The required amount of β-glucan was freshly dispersed in drinking water each morning, and the supplemented water was replaced every 24 h. Individual water intake was not measured; therefore, the amount of water delivered could not be expressed in mg/kg body weight/day.

2.4 Experimental design

The chicks were randomly allocated to five groups, with 60 birds in each, using a computer-generated randomization sequence. However, the personnel administering the treatments were not blinded to allocation of groups. The five groups were: Control (no vaccine, no β-glucan), Vac (vaccine only), and Vac β-Low, Vac β-Med, and Vac β-High (vaccine plus β-1,3/1,6-glucan at 0.5, 1 and 2 g/L, respectively). Each group had four replicate pens, with 15 birds per pen. The glucan-supplemented groups received the supplement on five consecutive days from day 6 to day 10, and again on days 21, 22, 28 and 29 to provide repeated short immunostimulatory exposures during the development of the primary antibody response before challenge at day 35. On day 7, all the birds, except those in the control group, were vaccinated with a single dose of live attenuated La Sota vaccine (CEVA, Egypt; CEVAC NEW L; batch number not recorded; at least 10^6 EID_{50} per dose) by eye drop and no booster was given (Fig. 1).

2.5 Growth performance

Individual body weight of birds was recorded on days 14, 21, 28, and 35 using a digital balance All available birds in each group at each

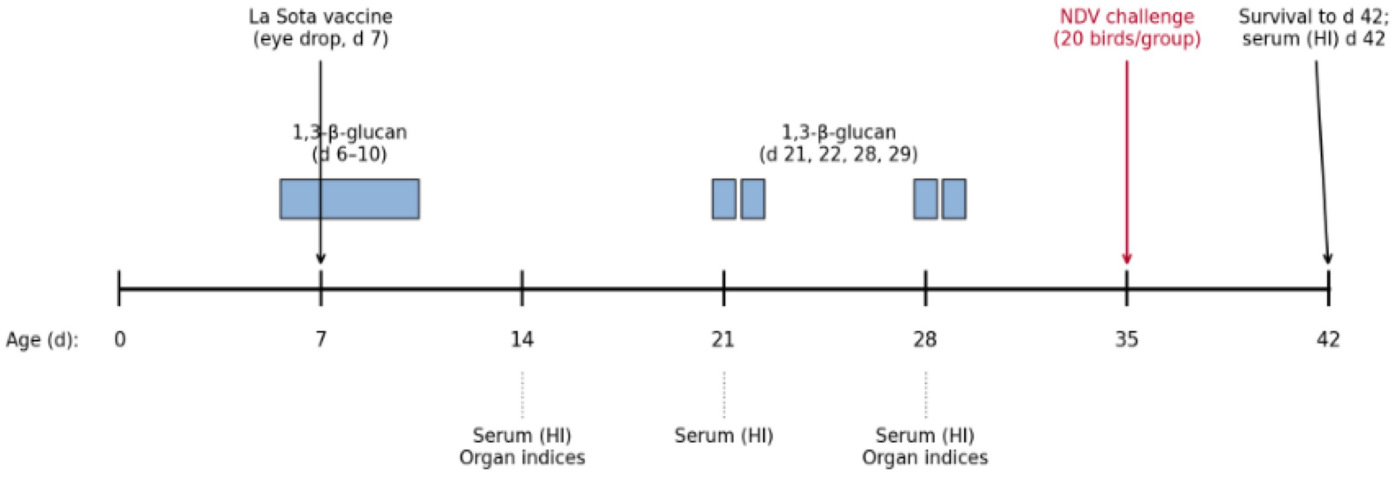


Fig. 1. *Timeline of the experiment. HI; hemagglutination inhibition; NDV: Newcastle disease virus*

sampling time were weight; however, number of available birds declined after six birds per group were removed on days 14 and 28. Body weight at placement was not recorded.

2.6 Immune organ indices

For organ sampling on days 14 and 28, six birds per group were randomly selected. One bird was selected randomly from each pen, and two additional birds were selected randomly from two pens at each sampling age, with the additional-sampled pens alternating between ages. The sampled birds were weighed and euthanized by intravenous sodium pentobarbital overdose after humane handling. The spleen, thymus, and bursa of Fabricius were removed and weighed. The relative organ weight was calculated as organ weight (g) per kg body weight.

2.7 Serology

Blood samples were collected from the wing vein of six birds per vaccinated group on days 14, 21, 28, and 42. Corresponding baseline sera samples were collected before initiation of β-glucan administration on day 6. Control birds were not sampled for HI. The HI tests used four hemagglutinating units of NDV antigen (La Sota) according to the WOAH Terrestrial Manual (WOAH 2021). The HI antibody titer was expressed as the reciprocal of the highest serum dilution that completely inhibited hemagglutination and was transformed to $\log_2$ scale for subsequent statistical analysis.

2.8 Virulent NDV challenge

On day 35, 20 birds per experimental group were challenged via oculonasal route with 10^6 EID_{50} of a virulent NDV strain (a locally isolated velogenic genotype VII NDV field strain with a multibasic fusion-protein cleavage site obtained from the institutional virus collection; ICPI was not determined in this experiment). The birds from each group were housed group-wise separately in biosecure isolation units. The clinical signs and mortality of the birds were observed daily for 7 days. Birds showing persistent recumbency, severe respiratory distress or moribundity, with inability to reach feed or water, were humanely euthanized and counted as dead). Survival on day 7 was the primary protection endpoint. On day 42, serum was collected from six surviving birds per vaccinated group for HI testing.

2.9 Statistical analysis

Continuous variables were summarized as the means ± standard errors (SE). The experimental unit was the pen for treatment delivery and growth; sampled birds were subsamples within pens for organ and serological endpoints. Because individual-level raw data and pen identifiers were not available, mixed-effects and pen-level sensitivity analyses could not be performed. The reported bird-level one-way analyses were therefore retained only as exploratory descriptions and may yield optimistic p values. Duncan's multiple range test does not control family-wise error; its letter groupings were reported as the original exploratory analysis and should not be treated as confirmatory. Separate statements were made for each age, and day-42 HI data were explicitly identified as survivor-only. Survival counts (n = 20 birds per group) were compared descriptively and by a Pearson chi-square omnibus test. Prespecified comparisons of each group with Vac used two-sided Fisher's exact tests with the Holm adjustment (Holm 1979). Odds ratios with exact 95% confidence intervals, Wilson intervals for proportions, and the difference in risk for Vac β-Med versus Vac are reported. A Cochran–Armitage trend test was applied across the vaccinated groups. Because each treatment occupied one challenge unit, these bird-level survival analyses cannot separate treatment effects from unit effects and are exploratory. No a priori sample size calculation was performed.

3. Results

3.1 Growth performance

Body weight data are presented descriptively in Table 1. A single overall F statistic is not appropriate for four ages, and the unusual day-21 to day-28 increase could not be verified or analyzed inferentially from the available aggregate records. Mean body weights on day 35 ranged from 2.0 kg (Control, Vac) to 2.2 kg (Vac β-Med, Vac β-High); however, no inferential conclusion is drawn from these values because raw individual and pen-level records were unavailable for reanalysis.

3.2 Immune organ indices

The relative spleen index was significantly higher in the Vac β-Med and Vac β-High groups than in the Vac group on day 14. On day 28, only the Vac β-High group differed significantly from the Vac group ($p < 0.05$; Table 2). The increase in spleen index was highest in the Vac β-High group on day 28 (3.0 vs 1.5 g/kg in Vac, a twofold difference), and the increase in the Vac β-Low group did not differ significantly from that in the Vac group. The bursa and thymus indices of the β-glucan supplemented groups did not differ significantly from those of the Vac group at either age. Compared with the control group, the bursa index was greater in the Vac β-Med and Vac β-High groups on day 14 and in the Vac β-Med group compared with control on day 28. The thymus index was significantly higher in all vaccinated groups at day 14 and in the Vac β-Med and Vac β-High groups on day 28 compared with the control ($p < 0.05$).

Table 1. Mean ± SE body weight (g) of the broiler chickens by age and group

Group	Day 14	Day 21	Day 28	Day 35
Control	400 ± 20	600 ± 30	1600 ± 60	2000 ± 80
Vac	300 ± 20	600 ± 30	1600 ± 50	2000 ± 70
Vac β-Low	400 ± 20	700 ± 30	1700 ± 60	2100 ± 80
Vac β-Med	400 ± 20	700 ± 30	1800 ± 60	2200 ± 80
Vac β-High	400 ± 20	700 ± 30	1800 ± 60	2200 ± 90

The values are presented as the mean ± SE. The marked increase between days 21 and 28 and the day-14 group pattern could not be independently verified from the available aggregate records. The values are retained descriptively and are not used for inference

Table 2. Relative weights of the spleen, bursa of Fabricius, and thymus (g /kg body weight; n = 6)

Group	Spleen d14	Spleen d28	Bursa d14	Bursa d28	Thymus d14	Thymus d28
Control	1.1 ± 0.1 ^{c}	1.3 ± 0.1 ^{c}	1.1 ± 0.1 ^{b}	2.0 ± 0.2 ^{b}	2.1 ± 0.2 ^{b}	3.0 ± 0.3 ^{b}
Vac	1.3± 0.1 $^{b c}$	1.5± 0.1 $^{b c}$	2.0± 0.2 ^{ab}	2.9± 0.2 ^{ab}	3.7 ± 0.3 ^{a}	4.2± 0.4 ^{ab}
Vac β-Low	1.5± 0.1 ^{ab}	1.5± 0.1 $^{b c}$	2.2± 0.2 ^{ab}	2.8± 0.2 ^{ab}	3.6 ± 0.3 ^{a}	4.2± 0.4 ^{ab}
Vac β-Med	1.8 ± 0.1 ^{a}	2.0 ± 0.2 ^{b}	2.4 ± 0.2 ^{a}	3.2 ± 0.2 ^{a}	4.0 ± 0.3 ^{a}	5.5 ± 0.4 ^{a}
Vac β-High	1.7 ± 0.1 ^{a}	3.0 ± 0.2 ^{a}	2.5 ± 0.2 ^{a}	3.0± 0.2 ^{ab}	4.1 ± 0.3 ^{a}	5.4 ± 0.4 ^{a}

The values are presented as the mean ± SE. Within each organ and age group, means without a common superscript differ at $p < 0.05$ according to Duncan's multiple range test

3.3 Serum antibody response

Baseline NDV antibody titers did not differ among the vaccinated groups, and the one-way ANOVA revealed no evidence of a group effect on day 14 across the vaccinated groups ($p \approx 0.93$; Table 3). On day 21, no statistically significant differences were observed among the vaccinated groups ($p \approx 0.08$). However, Duncan's unprotected multiple range test revealed a higher titer in the Vac β-Med group than in the Vac group ($p < 0.05$); whereas the titers of the Vac β-Low and Vac β-High groups were intermediate and did not differ significantly from that of the Vac group. Because Duncan's test does not control the family-wise error rate, the day-21 contrast should be read as hypothesis-generating rather than confirmed. On day 28, one-way ANOVA revealed no significance ($p \approx 0.22$) differences in the HI antibody titers across the groups. On day 42 (seven days after challenge), significantly higher ($p \approx 0.04$) titers were observed in Vac β-Med birds compared with Vac group (Table 3). The Vac β-High and Vac β-Low birds did not differ significantly from Vac and Vac β-Med birds. However, the sera came only from survivors, the interpretation of the comparison should be done cautiously challenge may have stimulated the antibody response and mortality may have introduced selection bias.

3.4 Protection against virulent NDV challenge

Unvaccinated control birds began to die 4 days after challenge, and 80% of birds died by day 7. Survival was 60% for Vac, 70% for Vac β-Low, 90% for Vac β-Med, and 80% for Vac β-High (Table 4, Fig. 2). Overall survival differed among the five groups ($\chi^2 = 25.4$, 4 df, $p < 0.001$) and was lower in the control group than in the vaccinated groups (unadjusted Fisher $p \leq 0.023$). Among the vaccinated groups, survival rate in the Vac β-Med group was 30 percentage points higher than that in the Vac group (odds ratio 5.7, 95% CI 0.9–64.6), but this difference was not significant according to Fisher's exact test ($p = 0.065$) and was further attenuated by the Holm adjustment ($p = 0.19$). Vac β-Low and Vac β-High did not differ from Vac. Survival did not increase significantly with increasing glucan dose among the vaccinated groups (Cochran–Armitage trend, $p = 0.12$), and the survival of the Vac β-Med group did not differ from that of the Vac β-High group (18/20 vs 16/20; Fisher $p = 0.66$).

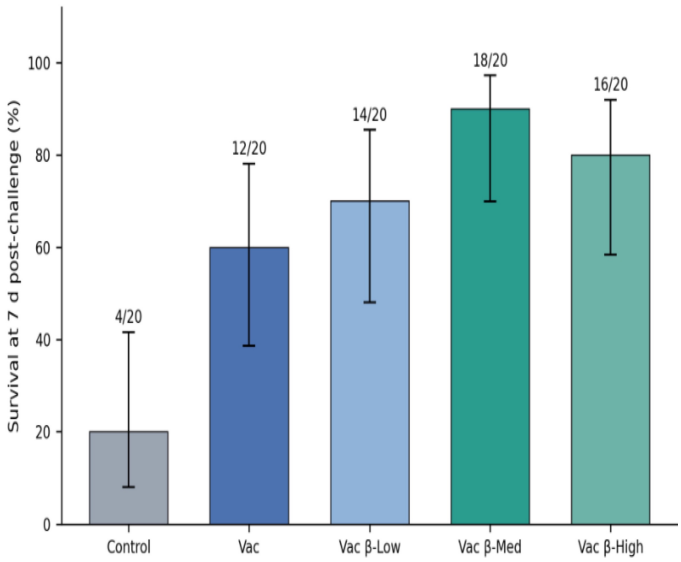


Fig. 1. *Survival rate 7 days after challenge. The bars show the percentage of surviving birds (n = 20) with Wilson 95% confidence intervals; the numbers above the bars are survivors/challenged*

Table 3. Serum HI antibody titers (log_2, mean ± SE; n = 6)

Group	Day 14	Day 21	Day 28	Day 42
Control	4.0 ± 0.37	4.7 ± 0.33 ^{b}	5.3 ± 0.33	6.0 ± 0.37 ^{b}
Vac	4.2 ± 0.31	5.2 ± 0.31 ^{ab}	5.5 ± 0.34	6.5 ± 0.34 ^{ab}
Vac β-Low	4.3 ± 0.33	6.0 ± 0.37 ^{a}	6.2 ± 0.31	7.5 ± 0.34 ^{a}
Vac β-Med	4.2 ± 0.31	5.5 ± 0.34 ^{ab}	6.0 ± 0.37	7.0 ± 0.37 ^{ab}
Vac β-High	0.93	0.08	0.22	0.04
Age-wise p value	0.93	0.08	0.22	0.04

The baseline titers measured on day 6 ranged from 3.5–3.7 log_2 across the vaccinated groups. At baseline, 10 of 24 sampled birds (42%) had titers ≥4 log_2; the proportion was 24/24 (100%) on days 21, 28 and 42

Table 4. Survival rates of broiler chicken seven days after challenge with virulent NDV (n = 20)

Group	Survivors	Survival (%) (95% CI)	Odds ratio vs Vac (95% CI)	Fisher p vs Vac	Holm-adjusted p
Control	4/20	20 (8–42)	0.18(0.03–0.8)	0.023	0.090
Vac	12/20	60 (39–78)	Reference	–	–
Vac β-Low	14/20	70 (48–85)	1.5 (0.4–7.1)	0.74	0.74
Vac β-Med	18/20	90 (70–97)	5.7 (0.9–64.6)	0.065	0.19
Vac β-High	16/20	80 (58–92)	2.6 (0.5–14.8)	0.30	0.60

Confidence intervals: Wilson (proportions) and exact conditional (odds ratios). Holm adjustment is across the four comparisons with Vac

4. Discussion

This exploratory dose-ranging study identified heterogeneous signals across endpoints. The 1 g/L group displayed a higher HI antibody titer on day 21 and numerically higher survival than the vaccine-only group, whereas the 2 g/L group displayed the highest spleen index on day 28. These findings do not demonstrate a coherent monotonic dose–response, an optimal dose, or improved protection. The challenge comparison is particularly limited because treatment was confounded with a single isolation unit per group, and the bird-level analysis therefore understates unit-level uncertainty. This pairwise contrast arose from Duncan's unprotected test; the corresponding omnibus comparison across vaccinated groups did not reach conventional significance on day 21 ($p \approx 0.08$), reinforcing that the finding is exploratory rather than confirmatory.

Survival was 90% in the Vac β-Med group and 60% in the Vac group. An unadjusted Pearson χ^2 test on these counts gives $p = 0.029$, which illustrates how sensitive the conclusion is to the test and to the absence of correction for the four comparisons; with Fisher's exact test and the Holm adjustment, it is $p = 0.065$ and $p = 0.19$. The confidence interval for the odds ratio (0.9–64.6) is compatible with both no benefit and a large benefit. Two design features further limit inference: challenge was applied to 20 birds per group, apparently without replicate units, so group and unit effects cannot be separated (Lazic 2010), and survival was the only postchallenge outcome. Clinical scoring, virus shedding and lesion or histopathological assessment, which are the outcomes that distinguish protection from disease from protection from infection (Kapczynski and King 2005), were not recorded. The lower survival of unvaccinated birds than the of birds in vaccinated groups suggests that the challenge produced considerable mortality. However, the design does not permit the separation of the contribution of vaccination from the potential unit and glucan effects. The direction of the effect is nevertheless consistent with earlier work. Combined β-glucan and mannan-oligosaccharide supplementation improved protection against virulent NDV challenge in Egyptian broilers (Tamam et al. 2010) and mushroom β-glucan dose-dependently protected against virulent NDV (Muthusamy et al. 2020). However, the results of present study should be read as a preliminary signal of the same kind rather than as independent confirmation.

Relative organ weights are crude and indirect indicators of immune status (Igwe et al. 2020). The spleen, a secondary lymphoid organ involved in antigen-specific immune responses, was the only organ that exhibited differences between β-glucan-supplemented groups and the vaccine-only group, and the maximum effect was observed at the highest dose on day 28. The bursa and thymus, which are primary lymphoid organs and mature largely independent of antigen exposure at this age, did not reveal differences among the vaccinated groups. However, higher values observed in the Vac β-Med and Vac β-High groups compared with the control group can be attributable at least partly to the vaccination itself. Approximately twofold increase in spleen weight at 2 g/L cannot be boldly interpreted without histology because lymphoid hyperplasia and inflammatory enlargement have different implications. Similar dissociation between antibody enhancement and immune organ development has been reported for dietary β-glucan in broiler chickens (Hashaam et al. 2024).

The HI antibody titer in the Vac β-Med group was significantly higher than the Vac group on days 21, but not on days 14 or 28. The HI titer in the Vac β-High group was not significantly different from that in the Vac group at any sampling time. The transient nature of the difference may reflect the timing of glucan courses (days 21–22 and 28–29) relative to sampling, the small number of birds sampled (n = 6), or true waning of the effect; however, the study design cannot discriminate among these. The absence of a day-14 difference is consistent with the interval needed for a primary response to a live vaccine administered at seven days of age, when residual maternally derived antibodies (MDA) may also have limited vaccine replication (Vrdoljak et al. 2017). Because maternal antibodies were not quantified and unvaccinated birds were not tested by HI, the extent of MDA interference cannot be evaluated. The higher day-42 titers in Vac β-Med survivors may partly reflect the selection of survivors and anamnestic responses to challenge virus and should not be interpreted as a vaccine effect. HI antibody titers are useful correlates of protection but do not necessarily explain the mechanism. Furthermore, cell-mediated and mucosal responses, which are important for protection against NDV and were improved by a yeast cell wall product in an earlier study (Bi et al. 2022), were not evaluated in this study.

Different doses ranked highest for different endpoints, but the doses did not differ significantly from one another in terms of survival (Vac β-Med vs Vac β-High, $p = 0.66$; trend $p = 0.12$) or bursa and thymus indices. The data shows no evidence that increasing glucan levels from 1 to 2 g/L provided additional benefits over 1 g/L. Also, the results are not sufficient enough to demonstrate a true inverted-U dose-response relationship. In contrast, Muthusamy et al. (2020) reported greater protection at higher doses and Hashaam et al. (2024) reported an effect only at the highest inclusion level. Thus, optimal dosing probably depends on the glucan source, structure, route, and schedule. Explanations such as receptor saturation or counterregulatory feedback at high ligand concentrations remain unclear because neither receptor engagement nor cytokine responses were measured. The effect of

dietary β-glucan on growth of broiler chickens in the present study could not be reliably evaluated because of unusual increase in body weight of birds from day 21 to 28, which could not be verified from the available records. Therefore, no conclusion regarding growth of broiler chicken with respect to β-glucan supplementation can be drawn. An earlier study has reported that dietary β-glucan did not improve daily weight gain in broiler chickens (Hashaam et al. 2024) but in contrast to another study reported higher body weight upon continuous supplementation (Tamam et al. 2010). Furthermore, the glucan supplementation in present study was done in short courses totaling nine days, which may be insufficient to influence the growth.

Potential mechanisms of β-1,3-glucan mediated enhancement of vaccine-induced immune responses include the effects of Dectin-1 and complement receptor 3 on antigen-presenting cells and trained immunity (Brown and Gordon 2001; Goodridge et al. 2009; Quintin et al. 2012; Netea et al. 2016). Orally administered glucan first contacts the gut-associated lymphoid tissue. However, the present study did not measure receptor expression, innate cell function or cytokines, and the oral route, live replicating vaccine, and the interval between glucan courses and vaccination differed from the mammalian settings in which these mechanisms were described. These mechanisms are therefore hypotheses for future work and not conclusions of this study. Although the administration of preparation through drinking water is operationally convenient; the actual glucan intake, dispersion uniformity, cost per bird, and effects on growth could not be determined. The 1 g/L group had a higher exploratory 21 day HI antibody titer, whereas the 2 g/L group had the higher 28 day spleen index. Whether this translates into field benefit will depend on the circulating genotype, vaccine–field virus match, MDA status, vaccination schedule, and cost per bird, none of which could be addressed here. In Egypt, where genotype VII viruses predominate, genotype-matched vaccines have been shown to improve protection against shedding (Sultan et al. 2020; Lebdah et al. 2024); an immunopotentiator should be viewed as a complement to, and not a substitute for, optimal vaccine selection and biosecurity.

5. Conclusions

Short term administration of a drinking water preparation containing approximately 80% β-1,3/1,6-glucan produced exploratory differences in selected immunological endpoints, but the patterns were not consistently dose dependent. The 1 g/L group displayed a higher HI antibody titer on day 21 and a numerically higher survival rate (90%) compared with with 60% in the vaccine-only group. The 42 day HI comparison was restricted to survivors and cannot be attributed solely to vaccination. The available aggregate growth records do not support inferential interpretation. Accordingly, the present study did not establish an optimal glucan concentration, improved protection, or an independent mechanism of immunostimulation. Therefore, confirmation requires prospective adequately powered studies with replicated challenge units, pen-aware analysis, verified intake, a glucan-only treatment group, systematic clinical scoring, evaluation of virological and pathological parameters, and prespecified immune endpoints.

Declarations

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Conflict of interest: The authors declare that they have no competing interests

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Data availability statement: The relevant data will be available from corresponding author upon reasonable request

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