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Administration of Beta glucan on humeral immunity after Foot and mouth disease vaccine in Local Awassi Male Lambs

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Abstract

This study aimed to investigate the stimulating impact of orally given β-glucan extracted from brewing yeast Saccharomyces cerevisiae on leukocyte functions and immunization responses in lambs. Ten healthy male awassi lambs (weighting an average of 18.2 kg) were divided into two groups (A and B), each with five animals, and given the foot-and-mouth vaccination. Lambs from group A received 4 mg/kg of beta glucan (β-glucan) orally once daily for 60 days after vaccinated with (ARRIAH-VAC) intramuscularly in the neck at a dose of 1 ml while another group B consider as control group that vaccinated with (ARRIAH-VAC) intramuscularly in the neck at a dose of 1 m . Blood was drawn from the jugular vein at the start of the trial (day 0), as well as on days 15, 30, and 60 of the experiment to evaluate the chosen markers of humoral immunity in lambs. After immunization, ELISA was used to assess antibody titers against FMD vaccine types A,O and Asia with significant (P>0.05) at days 15 and 30. Our findings show that oral administration of beta glucan following FMD vaccinations can overcome antibodies to induce significant and strong antibody responses in growing lambs.

Keywords: *Beta glucan, FMD vaccine (ARRIAH-VAC), ELISA.*

Introduction

The serious infectious viral disease known as foot-and-mouth disease (FMD) primarily affects animals with cloven hooves (Alexandersen et al., 2003), Pathogenic bacteria, algae, barley and yeast are the most prevalent sources of the polysaccharides known as glucans. (Akramienė et al., 2007; Geng et al.,2022; Reis et al.,2022), Major structural elements of the cell walls of yeast and fungi are glucans with 1,3 and 1,6 glycosidic connections (β-glucan) (Jorgensen and Robertsen,1995). β-glucan has been shown to have anticancer properties (Divya et al.,2020; Liao et al.,2022) as well as antibacterial activities via enhancing host immunological response (Mahdi and AL-Abass, 2012; Milewski et al.,2013;Hassana et al.,2014; Mahdi and Mohsin, 2015), furthermore β -glucans are a helpful tool for ruminant producers as an alternative to antibiotics (Krüger and Van Der Werf , 2018; Medina-Córdova et al.,2018), in addition antioxidant characteristics of β-glucan and the capacity of β-glucan to repair cell damage (Al-faragi et al., 2014; Oliveira et al.2007), additionally according to reports, myeloid cells in mammals have multiple receptors that recognize β-glucans (Goodridge et al., 2011;Taylor et al., 2007), moreover β-glucans acts as an immunomodulator, increasing nonspecific immunity (El-Boshy et al.,2010; Schwartz and Vetvicka 2021), as well as reducing stress caused by the environment (Anwar et al.,2007; Mahdi and Naser, 2014), besides that capable of increasing antigen-specific and nonspecific IgA production in a variety of mucosal locations (Dos Santos et al.2023; Hashimoto et al.,1991; Tanioka et al.,2013) and

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increases lymphocyte proliferation (Jabbar et al.,2013) as well as the growth-stimulating actions of the antioxidant substance (β-glucan) may potentially be connected to its antioxidative function, which prevents free radicals (ROS) (Alhasnawi et al.,2016; Caruso , et al 2022; Machová and Bystrický, 2013).The aim of this study to investigated the impact of oral glucan supplementation on serum antibody response in healthy lambs following FMD vaccine immunization (FMDV).

Material and methods

Location, Date, and Lambs Management of the Experiment

This study was carried out in the Field-College of Veterinary Medicine/University of Baghdad from January 9, 2022, until April 30, 2022. Before the study started, each lamb received a number using ear tags and being under veterinarian supervision. Clinical safety is continuously and carefully evaluated by the preventative system. Every preventative measure was taken for all lambs, including a 21-day adaptation period to the farm environment and a subcutaneous (s/c) injection of ivermectin (0.5ml/lamb) to protect against external parasites. All lambs were administered oral doses of the anthelmintic worminex (2ml/lambs), which were repeated after two weeks, to protect them against the consequences of internal parasite infection.

lambs: care, feeding, and treatment

The Local Ethics Committee for Animal Utilization Protocol gave their approval to all of the study's procedures involving the animals, with an average body weight of 18.656 kg, 10 healthy local male Awassi lambs were obtained at the age of around 3–4 months and housed in the animal field of the University of Baghdad's Veterinary College which were maintain in the same condition . The animals received green alfalfa, hay, and tap water for a preliminary period of three weeks after that the lamb vaccinated with (ARRIAH-VAC) intramuscularly in the neck at a dose of 1 ml. The research is 12 weeks long, and body weight was taken into account. The animals were housed in cages that were made for lambs and kept tightly locked. The animals were divided routinely and equally into two groups, each of which contained five lamb. The first group (G1) was kept as the control group and received daily feedings of concentrated diet amounting to 2.5% of body weight (Table 1); however, the second group (G2) received oral administration of beta glucan at a dose of 4 mg/kg (Vojtek et al ,2017) once daily for eight weeks.

Table 1: Composition of concentration diet's ingredients:

nutritional ingredients	**%**
Wheat bran	20
Soya bean	10
Barley	48
Corn	20
Salt	1
premix	1
% Total	100

Extraction of -glucan from Saccharomyces cereviseae cells

Extraction methods done according to (Jameel and Yassein, 2021) briefly 225 g of dried yeast and 1.5 L of 1M NaOH were combined, and the mixture was stirred at 80 oC for two hours. The cell pellet was then recovered by cold centrifugation at 6000 x g for 25

min. at 4 oC and suspended in three folds of distilled water. Repeating the procedure led to the supernatant being discarded and the pellet being taken to dissolve in (1.5) L of 1M acetic acid (CH3COOH), followed by a 2-hour incubation period in a magnetic stirrer at 80 oC. the pellet was then collected by centrifugation at (6000xg) for 25 min. at 4 oC, washed three times with water, and centrifuged at (6000xg) for 25 min. at 4 oC. Following that, the pellet was combined with 600 ml of 100% ethanol using a magnetic stirrer for 1 hour. The suspension was then centrifuged at 6000 x g for 25 minutes at 4 oC, and the pellet was dried in a hot oven for 24 hours at 60 oC.

Serum sample collection

On days 0 through 14, 30, 45, and 60 of the growth phase, blood samples were taken from the lambs' jugular veins. The serum from the blood was separated by centrifuging it at 3000 g for 20 minutes; it was then decanted and refrigerated (at -20 °C) for further analysis.

ELISA Test

Three flat-bottomed ELISA plates were coated with 50 µl of the immune rabbit serum (IRS) against O IND R2/75, A IND40/2000, and Asia1 IND 63/1972 for each serotype. Plates were then kept at 4°C overnight for incubation. They were serially diluted in PBS from 1:4 to 1:512 for the test sera and positive control (serotype-specific Bovine Vaccinated Serum [BVS]). Then, in each well of the specified plates for each serotype, 50 µl of diluted antigens were added, with the exception of the wells in the 12th column. The 10th column served as a positive control, the 11th column an antigen control, and the 12th column remained empty. The plates were then incubated at 4°C for the next day The contents of the flat-bottomed ELISA plates were thrown away, and the plates were then washed five times with 200 µl of PBST. A 50 µl portion of the Ag-Ab mixture that had been incubated was transferred from the 'U' bottomed plates to the corresponding wells of the flat bottomed ELISA plates. After one hour of 37°C incubation, the plates were washed. Each type-specific plate received 50 µl of reconstituted serotype-specific tracer immune guinea pig serum (IGPS), which was added, incubated at 37 °C for an hour before the plates were washed. A 50 µl substrate solution (OPD activated with 30–33% hydrogen peroxide solution) was added to each well of the plates after they had been cleaned. In a dark environment, 50 µl of substrate solutions (OPD activated with 30- 33% Hydrogen peroxide solution) were added to each well after washing the plates For 15 minutes, all of the plates were incubated at 25 °C. By adding 50 µl of stopping solution, the color reaction was stopped (1.25M Sulphuric acid). The optical densities of the wells were measured at 492 nm with an ELISA plate reader and the Magellan program. The maximum dilution that resulted in a 50% reduction in OD value when compared to the antigen control wells was used to determine the serum sample's titer.

Statistical analysis

a result denoted by its mean and standard error. Data statistical analysis was performed using SAS (Statistical Analysis System - version 9.1). Using one-way ANOVA and the Least Significant Differences (LSD) post hoc test, the significance of differences between means was assessed. (P> 0.05) denotes statistical significance (SAS,2018).

Result

Antibody titer data from the FMD vaccination type A that demonstrate the humoral response When compared to the same period in groupA, the significant (P> 0.05) difference in Ab titers at 14, 30, 45, and 60 days old while in group B demonstrated a decrease in Ab titers for all type of FMDV as shown in table 2.

Table 2: shows the impact of β-glucan oral administration (4 mg/Kg BW) on the antibody titer for the (type A) FMD vaccination for each group.

Ab titer (type A FMDVaccine) Elisa test	**(Group A) β-glucan group**	**(Group B) Control group**	**LSD**
Zero time	D0.11±0.01a	C0.15±0.01a	0.1
After 14 of FMDV	A0.49±0.05b	A0.57±0.03a	
After 30	BC0.35±0.05ab	B0.24±0.03b	
After 45	C0.33±0.06b	B0.29±0.02b	
After 60	C0.33±0.05b	B0.32±0.02b	

Means with a different small letter in the same raw are significantly different (P<0.05)

Means with a different capital letter in the same columnare significantly different (P<0.05)

Results of the Elisa test used to evaluate antibody titers to FMD vaccine type O at 14, 30, 45, and 60 days following immunization are displayed in (Table ,3).

Table 3: shows the impact of β-glucan oral administration (4 mg/Kg BW) on the antibody titer for the (type O) FMD vaccination for each group.

Ab titer (type O FMDVaccine) Elisa test	**β- glucan (A)**	**Control(B)**	**LSD**
Zero time	D0.11±0.01a	D0.14±0.01a	0.11
After 14 of FMDV	A0.58±0.02a	B0.45±0.03a	
After 30	B0.45±0.03a	C0.26±0.04a	
After 45	C0.28±0.05b	BC0.29±0.03ab	
After 60	BC0.34±0.06ab	BC0.31±0.02b	

Means with a different small letter in the same raw are significantly different (P<0.05)

Means with a different capital letter in the same columnare significantly different (P<0.05)

Table 4 displays the immunoglobulin G serum levels against FMD vaccine type Asia. The serum levels of IgG was affected by -glucan supplementation (P>0.05) type.

Table 4: shows the impact of β-glucan oral administration (4 mg/Kg BW) on the antibody titer for the (type ASIA) FMD vaccination for each group.

Ab titer (type ASIA FMDVaccine) Elisa test	β- glucan (A)	Control(B)	**LSD**
Zero time	D0.11±0.01a	C0.15±0.01a	0.11
After 14 of FMDV	A0.65±0.05a	A0.66±0.04a	
After 30	BC0.43±0.03b	BC0.55±0.03a	
After 45	B0.54±0.03a	BC0.47±0.04ab	

After 60	0.38±0.04a	C0.37±0.03a	

Means with a different small letter in the same raw are significantly different (P<0.05)

Means with a different capital letter in the same columnare significantly different (P<0.05)

Discussion

A number of clinical investigations on the effects of oral beta-glucan formulations have been done in recent years (Van Steenwijk et al,2021; Kaminski et al.,2021; Preethy et al.;2022). Yeast and fungal glucans have one to three bonds (Moreno-Mendieta et al.,2017; Osmond et al.,2001; Ruiz-Herrera and Ortiz-Castellanos,2019) and depending on the kind of glucan, they may also have one to six extra branches. Most barley and oat glucans that have been isolated are linear compounds made up of regions with 1-4 bonds that divide smaller structures with 1-3 bonds (Jobling ,2015; Lazaridou et al.,2004). According to the findings of lamb research, β-1,3/1,6-D-glucan, a structural component of the cellular wall of Saccharomyces cerevisiae, plays an essential part in this process in terms of markers of both non-specific humoral immunity and innate immunity (Bilal et al.,2022; Jergens and Heilmann 2022;). The current study looked at the effects of orally given beta-glucan on lamb immunization responses to FMD vaccine, due to the fact that glucans are found in microorganisms, they are categorized as pathogen-associated molecular patterns that may be identified by pattern recognition receptors on host immune cells and hence cause an immunological response (Volman et al.,2008; Wojcik,2010). It is possible for β-glucan to reduce environmental stress, and this action may enhance immunity (Mahdi and Naser,2012) and enhanced serum activities (Ching et al.,2021; Lei et al.,2015; Ringø and Song 2016). As a result, they are referred to as immunomodulating chemicals (Samuelsen et al., 2014). The main finding of this study was that providing beta glucan gradually had an overall better influence on lambs' average daily growth (El Khoury et al.,2012; Reilly et al.,2010; Song et al.,2021) and enhance immune respose and stimulate lymphocyte proliferation and enhance humoral immune response (Abdelhamid et al.,2020; An et al., 2008; Koch et al,2021; Ogier de Baulny et al.,1996). This result was consistent with past research using other feed additives (Dawood and AL-Saigh, 2014; Dawood, 2014, Al-Absawi et al.2020; Kadhim, and Dawood2020) which was used to arrive at the same conclusion.

Conclusion:

The findings showed that supplementing with β-glucan raised the serum FMD immunoglobulin titer and increased body weight gain in awassi lamb.

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