β-glucan administration improves growth performance and gut health in New Zealand White and APRI rabbits with different breed responses

Mahmoud M. Abo Ghanima 1, Ayman H. Abd El-Aziz 1, Ahmed E. Noreldin 2, Mustafa S. Atta 3, Shaker A. Mousa 4, Ali H. El-Far 5*

1 Department of Animal Husbandry and Animal Wealth Development, Faculty of Veterinary Medicine, Damanhour University, Damanhour, Egypt, 2 Department of Histology and Cytology, Faculty of Veterinary Medicine, Damanhour University, Damanhour, Egypt, 3 Department of Physiology, Faculty of Veterinary Medicine, Kafrelsheikh University, Kafrelsheikh, Egypt, 4 Pharmaceutical Research Institute, Albany College of Pharmacy and Health Sciences, Rensselaer, NY, United States of America, 5 Department of Biochemistry, Faculty of Veterinary Medicine, Damanhour University, Damanhour, Egypt

* ali.elfar@damanhour.edu.eg

Abstract

This study investigated the effects of oral administration of β-glucan 1,3 (pharmaceutical grade 10%) on growth performance and carcass traits in two breeds of weanling rabbits adapted to survive in Egypt, New Zealand White (NZW) and Animal Production Research Institute (APRI) rabbits, with special attention to relative mRNA expression of interleukins and antioxidant enzyme genes, biochemical, and histological alterations. Oral administration of β-glucan with doses 0.25 and 0.5 ml per one-liter of drinking water significantly accelerated body weight gain (BWG) in both rabbits' breeds, reduced total feed consumption (FC), and reduced feed conversion ratio (FCR), especially the 0.5 ml per one-liter dose in both rabbit breeds. There are remarkable differences in all the growth performance traits due to breed effect. The interaction effect between β-glucan and breed significantly improved BWG, FC, and FCR. There were non-significant differences in all carcass traits studied due to oral administration of β-glucan with both doses, except in dressing percentages. The highest of the dressing percentages were observed at doses 0.25 ml per one-liter (51%) and 0.5 ml per one-liter (52%) compared with control (50%). Our findings show significant variations in the final BW, total daily gain, feed consumption, and total feed conversion ratio between NZW and APRI rabbits. Absence of significant differences in the hot carcass weight and dressing percentage between the genetic groups had been reported in this study. Supplementing NZW and APRI rabbits with β-glucan increased blood total protein and globulin. The duodenal villi dimensions, splenic lymphoid diameter, muscular fiber diameter, and muscular glycogen areas were significantly increased by β-glucan administration. Expression of intestinal interleukin-18 (IL-18) in NZW rabbits treated with 0.25 and 0.5 doses of β-glucan was significantly upregulated and enhanced the immune response. β-glucan upregulated the expression of intestinal occludin mRNA particularly at dose 0.5 β-glucan as well as upregulated intestinal superoxide dismutase 1 (SOD1) and glutathione...
peroxidase 1 (GPx1), which modulates anti-inflammatory and antioxidant properties. In conclusion, oral administration of β-glucan at a dose of 0.25 or 0.5 ml per one-liter drinking water provided beneficial effects in the growth performance and health status of rabbits.

Introduction
Rabbits meat production is a practical solutions to the growing protein shortage in developing countries [1]. In many European and North African countries, including Egypt, meat is consumed routinely and its production plays a major role in most of those countries’ economies [2]. To help resolve the global protein shortage problem, production of rabbits is an appropriate task due to high fertility, low investment costs, a short interval between generations, and the ability to use various forages [3]. Rabbit meats are also highly digestible, delicious, and low-calorie foods that nutritionists often recommend over other meats [4] because rabbit meat is about 20% proteins, unsaturated fatty acids, potassium, phosphorus, and magnesium along with low contents of fat, cholesterol, and sodium [5].

The European Union prohibition of the use of antibiotic growth promoters led to research for various natural feed additives rather than food antibiotics including probiotics, prebiotics, enzymes, and organic acids [6]. A natural feed additive is β-1,3–1,6-glucan, the structural constituent that is present in the cell wall of yeast, fungi, and certain bacteria [7]. β-1,3–1,6-glucan can be supplied as alternate feed additive orally and is absorbed by intestinal cells and intestinal lymphoid tissue cells into the gastrointestinal tract, stimulating molecular and humoral immune reaction cells [8]. Advances were noted in immunity by supplementing β-1,3–1,6-glucan in rabbits [9] chicken [10], swine [11], and horse [12].

The current research was therefore carried out to explore the impacts of oral administration on the growth and carcass characteristics, with specific attention paid to their molecular, biochemical, and histopathological changes, in New Zealand White (NZW) and Animal Production Research Institute (APRI) rabbits, two races of weaning rabbits adapted to survive in Egypt.

Materials and methods
Ethical statement
The research was endorsed by the Faculty of Veterinary Medicine (Damanhour University, Egypt), committee of Local Experimental Animal Care. During the experiment, all precautions were taken to reduce the animal suffering.

Animals, management, and the experimental design
A new maternal line (APRI) established from Egyptian Baladi Red (BR) and a Spanish line (V) rabbits was started in 2002 at the Sakha experimental rabbity, Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture, Egypt. The APRI line was established by crossing Baladi Red bucks with V line does to produce F1 (½B½V) stock, followed by two generations of inter se matings to achieve performance stability. Rabbits of both breeds at the 6th week of age and 680±40 g body weight were allotted randomly into 6 groups (20 rabbits per each). This experiment was carried out at a private farm on these 120 weaned male rabbits of 6 weeks of age (680±40 g live body weight). Animals were allotted into a
completely randomized design in a 2 × 3 factorial arrangement (two breeds: NZW and APRI, and three levels of ß-glucan (ßG): 0 (control), 0.25, and 0.5 ml per one-liter drinking water).

Rabbits were reared in a semi-closed rabbitry of 180 m² (6 m width and 30 m length) with wire-netted windows in eastern and western sides for natural ventilation. Windows were oriented with an elevation of 160 cm from the floor, which was concrete with moderate slope to middle to facilitate drainage of water and waste liquids towards large gutters to the outside. During cold, windy weather and at night the windows were closed for protection from severe atmosphere.

Rabbits were housed in galvanized wire batteries with standard dimensions (60 x 35 x 35 cm). All cages were supplied with galvanized-steel feeding hoppers and automatic drinkers (nipples). Rabbits were identified by plastic ear tags. Fresh water was offered ad libitum. Rabbits were fed on a standard pelleted ration offered ad libitum twice daily at 8 am and 2 pm. The pellets were 1 cm length and 0.4 cm diameter. Rabbit cages were regularly cleaned and disinfected. Urine and feces dropped beneath the batteries were removed every day in the morning.

Rabbits from each breed were allocated into 3 groups (20 rabbits each) with one group considered as a control. The treated groups received ß-glucan 1,3 pharmaceutical grade 10% concentration at a dose of either 0.25 ml or 0.5 ml per one-liter of drinking water for 3 successive days each week. Each individual rabbit in 0.25 ml ß-glucan-treated group was supplemented with 233.25 mg of ß-glucan during 10-week experimental period, while in 0.5 ml ß-glucan-treated group each rabbit was supplemented with 466.5 mg of ß-glucan. Modulin Plus® (Micro-Biotech Company, Miami, FL, USA) was used as a source of ß-glucan 1,3 pharmaceutical grade (10%).

**Experimental diet**

The basal experimental diet was formulated following the NRC [13] and de Blas and Mateos [14] recommendations and then pelleted to satisfy the nutrient requirements of rabbits (Table 1). Ingredients needed for formulation of the experimental diets were finely ground by using hammer mill screen size 3.0 mm, then weighing of different ingredients at required amount for the experimental diets, thoroughly mixed and pelleted (3.5 mm size).

**Growth performance traits**

Rabbits were individually weighed at the beginning (6th week) and at the 16th week of age, then daily weight gain was calculated during the whole period. Weighing was done in the early morning before rabbits received any feed or water. Feed consumption per rabbit was recorded daily. Residues and wasted feed were weighed daily and then subtracted from the offered amounts to obtain the actual accumulated feed consumed, and then the feed conversion ratio (FCR) was calculated. Also, body weight (BW), body weight gain (BWG), and total feed conversion (FC) were determined [15].

**Carcass traits**

At the 16th week, 3 representative rabbits from each group were randomly taken to estimate the carcass traits. Rabbits were fasted for approximately 6 hours before sacrifice and then individually weighed. Carcass was eviscerated after skinning, and giblets (liver, heart, and kidneys) were removed and weighed to determine the dressed weight and the dressing percentage. All data were recorded as percentage to the live body weight [16].

Dressing percentage was calculated as (hot carcass weight × 100/fasted weight). Carcass was separated for the following three cuts: (1) two fore legs (including thoracic insertion muscles),
(2) loin (including the abdominal wall and the ribs after the 7th thoracic rib), and (3) hind legs (including the sacral bone and the lumber vertebra after the 6th lumber vertebra).

Biochemical assessments
After sacrifice, blood samples (n = 5 for each group) were collected and then tubes were left in slope position until serum samples were separated through centrifugation at 1000 ×g for 20 minutes. The collected sera were subjected to biochemical analyses.

Serum total protein, albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, and urea were determined using commercial kits according to the manufacturers’ instructions (Bio-diagnostic, Giza, Egypt). Serum globulin concentration was calculated by the difference between total protein and albumin, and the albumin/globulin ratios were calculated.

Histomorphometry
Five samples from five different rabbits of each group of one cm in length were sliced from duodenum, spleen, and pectoral muscle preserved in 4% paraformaldehyde dissolved in PBS. Then,
tissues were prepared using the standard histological technique including dehydrating with ascending percentage of ethanol until reaching 100% ethanol. Then, cleared in xylene and melted paraffin ended by embedding in paraffin wax at 65°C. The paraffin blocks were sectioned at 4μm thickness using a microtome, then these sections were stained with Hematoxylin and Eosin (H&E) and for periodic acid schiff (PAS) according to the method of Bancroft and Layton [17].

From each intestinal segment, three sections were used (one section from serial 10 sections). From every section, 5 complete villi having perfect orientation and intact lamina propria were selected indiscriminately for inspection. Therefore, an average of 15 values were obtained for each intestinal sample. Slides were examined under a light microscope (Leica DM500, Leica, Germany) at 4X magnification, supported with a digital camera (Leica EC3). Images were analyzed with an image processing system photo analyzer (Image J; v1.46r, NIH, Bethesda, MD, USA) as described by Schneider et al (2012). The variables calculated for histomorphological modulations were crypt depth (CD), villus height (VH), villus width (VW), and villus height to crypts depth ratio (VH: CD) according to the method of Saeed et al [18] and Kiczorowska et al [19].

Well-oriented germinal center areas in the spleen were combined together and were noted as a percentage of the total field of view at 4X magnification using a Leica light microscope (Madej et al., 2015) and measured as optical denisty of splenic white pulp by (Image J). Later the average of 3 sections values was determined.

Cross dissections of pectoral muscle were processed, sectioned, and stained for quantification of mean fiber cross-sectional area as previously described Heywood et al [20], and the glycogen area was evaluated according to the protocol of Prats et al [21]. Light photomicrographs at 40X magnification were taken using a Leica light microscope and images were analyzed using Image J.

**Assessment of gene expression**

Total RNA was obtained from the samples (n = 5 for each group) using easy-RED Total RNA Extraction Kits (iNtRON Biotechnology, Inc., Korea) as directed by the manufacturer. Agarose gel electrophoresis was used to check the integrity of RNA, and a NanoDrop spectrophotometer was used to analyze the quantities and purities of the samples. First-strand cDNA was obtained using a kit for HiSenScript cDNA (iNtRON Biotechnology, Inc., Korea). Specific primers were used to amplify chosen genes with GAPDH as a housekeeping gene that was stable among the sample groups (Table 2). The mRNA expression was performed using a Stratagene MX3005P real-time PCR (Agilent Technologies, CA, USA) and TOPreal™ PreMIX SYBR Green qPCR master blend (Enzynomics, Daejeon, Republic of Korea) following the suggestions of the manufacturer. MxPro QPCR Software was used. The relative concentrations of gene expression were assessed using the $2^{-ΔΔct}$ technique as outlined in Pfaffl [22].

**Statistical analysis**

The body weight data were normally distributed and subjected to statistical analysis using Two-way analysis of co-variance for initial body weight data; the general linear model (GLM) of the SAS program (SAS Institute, SAS® 2009). The following model was fitted: Yijkl = μ + Wi + Sj + Ek + SEjk + eijkl, where Yijkl = observed value of the concerned treatment, μ = observed mean for the concerned treatment, Wi = effect due to covariance of the initial weight, Sj = effect due to breed, Ek = effect due to β-glucan, SEjk = interaction effect due to breed and β-glucan, and eijkl = the error related to individual observation. While, the weight gain, feed consumption and feed conversion data were normally distributed and subjected to statistical analysis using Two-way analysis of variance for initial body weight data; the general linear model (GLM) of the SAS program (SAS Institute, SAS® 2009). The following model was fitted:
Yijk = μ + Si + Ej + SEij + eijk, where Yijk = observed value of the concerned treatment, μ = observed mean for the concerned treatment, Si = effect due to breed, Ej = effect due to β-glucan, SEij = interaction effect due to breed and β-glucan, and eijk = the error related to individual observation. Differences between means were tested with Duncan’s multiple range test at the level of α = 0.05 [23]. The percentages of the studied traits were transformed to Arcsine values and then re-transformed to the original values after analysis. Statistical analysis of gene expression data was done with one-way ANOVA and Tukey’s post hoc test for multiple comparisons using with GraphPad prism 5 (San Diego, CA, USA).

Results

Growth performance

Results of growth performance (BW, BWG, FC, and FCR) are presented in Table 3. Oral administration of β-glucan at doses 0.25 and 0.5 ml per one-liter drinking water significantly (P < 0.05) accelerated BW in rabbits and reduced FCR in comparison with control. The 0.5 ml per one-liter drinking water β-glucan administration was the best dose for rabbits. There is a remarkable difference in all growth performance traits due to breed effect. The interaction effect between β-glucan and breed was significant on BWG, FC, and FCR and the highest gain and the lowest FCR were noticed in each breed when interacted with β-glucan (Table 3).

Carcass traits

Findings of carcass traits showed non-significant differences in all carcass traits studied for oral administration of β-glucan, breed, and their interaction (Table 4), except in forequarters,
Table 3. Growth performance of rabbits as affected by breed and β-glucan administration.

| Items                  | Final body weight (g) | Body weight gain (g) | Total feed consumption (g) | Feed conversion ratio (g feed/g gain) |
|------------------------|-----------------------|----------------------|-----------------------------|---------------------------------------|
| **Breed effect**       |                       |                      |                             |                                       |
| NZW                    | 2598.84 a             | 1819.00 a            | 5396.33 b                   | 3.155 b                               |
| APRI                   | 2383.16 b             | 1726.00 b            | 6072.33 a                   | 3.375 a                               |
| SEM                    | 4.86                  | 8.47                 | 0.289                       | 0.016                                 |
| P value                | 0.001                 | 0.001                | 0.001                       | 0.001                                 |
| **β-glucan administration** |                     |                      |                             |                                       |
| βG0.25                 | 2479.71 b             | 1769.50 b            | 5718.00 b                   | 3.230 b                               |
| βG0.5                  | 2672.97 a             | 1939.00 a            | 5422.50 c                   | 2.794 c                               |
| Control                | 2320.31 c             | 1609.00 c            | 6062.50 a                   | 3.770 a                               |
| SEM                    | 4.86                  | 8.47                 | 0.289                       | 0.016                                 |
| P value                | 0.001                 | 0.001                | 0.001                       | 0.001                                 |
| **Breed × treatment interactions** |                     |                      |                             |                                       |
| NZW Control            | 2255.05 f             | 1599.00 d            | 5853.00 c                   | 3.664 b                               |
| βG0.25                 | 2345.84 e             | 1692.00 c            | 6272.00 a                   | 3.876 a                               |
| βG0.5                  | 2548.58 d             | 1887.00 b            | 6173.00 b                   | 3.348 b                               |
| APRI Control           | 2385.57 d             | 1619.00 c            | 6272.00 a                   | 3.876 a                               |
| βG0.25                 | 2613.57 b             | 1847.00 b            | 6173.00 b                   | 3.348 b                               |
| βG0.5                  | 2797.36 c             | 1991.00 a            | 5772.00 d                   | 2.900 a                               |
| SEM                    | 4.86                  | 8.47                 | 0.289                       | 0.001                                 |
| P value                | 0.001                 | 0.001                | 0.001                       | 0.001                                 |

Means within each column for each division with no common superscript letters are significantly different (P < 0.05).

SEM = standard error of means. βG = β-glucan

https://doi.org/10.1371/journal.pone.0234076.t003

Table 4. Carcass traits of rabbits as affected by breed and β-glucan administration (%).

| Items                  | Forequarter | Loin | Hindquarter | Giblets | Dressing |
|------------------------|-------------|------|-------------|---------|----------|
| **Breed**              |             |      |             |         |          |
| NZW                    | 0.327       | 0.282| 0.400       | 0.056   | 0.513    |
| APRI                   | 0.325       | 0.272| 0.399       | 0.050   | 0.310    |
| SEM                    | 0.004       | 0.004| 0.003       | 0.003   | 0.002    |
| P value                | 0.81        | 0.19 | 0.76        | 0.25    | 0.38     |
| **β-glucan treatment** |             |      |             |         |          |
| βG0.25                 | 0.325       | 0.280| 0.402       | 0.056   | 0.51 a   |
| βG0.5                  | 0.332       | 0.281| 0.405       | 0.051   | 0.52 a   |
| Control                | 0.322       | 0.269| 0.391       | 0.050   | 0.30 b   |
| SEM                    | 0.004       | 0.004| 0.003       | 0.003   | 0.002    |
| P value                | 0.52        | 0.32 | 0.13        | 0.58    | 0.002    |
| **Breed × treatment interactions** |         |      |             |         |          |
| NZW Control            | 0.320 a     | 0.270| 0.383 b     | 0.055   | 0.504 b  |
| βG0.25                 | 0.329 a     | 0.280| 0.400 ab    | 0.056   | 0.514 ab |
| βG0.5                  | 0.332 a     | 0.295| 0.419 a     | 0.057   | 0.322 a  |
| APRI Control           | 0.315 b     | 0.267| 0.392 b     | 0.045   | 0.500 b  |
| βG0.25                 | 0.317 ab    | 0.281| 0.399 ab    | 0.048   | 0.510 ab |
| βG0.5                  | 0.344 a     | 0.268| 0.405 ab    | 0.056   | 0.520 a  |
| SEM                    | 0.004       | 0.004| 0.003       | 0.003   | 0.002    |
| P value                | 0.05        | 0.21 | 0.02        | 0.71    | 0.05     |

Means within each column for each division with no common superscript letters are significantly different (P < 0.05).

https://doi.org/10.1371/journal.pone.0234076.t004
hindquarters, and dressing percentages ($P < 0.05$) due to the interaction between β-glucan and breed. The highest percentages of forequarters, hindquarters, and dressing percentages were obtained from NZW when administered with 0.5% β-glucan (33.2%, 41.9%, and 52.2%, respectively).

### Biochemical analyses

Administrating rabbits with β-glucan at a dose 0.5 ml per one-liter drinking water increased blood total protein and globulin values (Table 5). β-glucan significantly increased uric acid in comparison with control, while urea and creatinine levels were non-significantly changed.

### Histomorphometry

Mucosal histomorphometric studies revealed significantly ($P < 0.05$) higher VH in duodenum of groups treated with 0.5% β-glucan compared with control (Table 6) and (Fig 1). Moreover, higher VH:CD ratio was observed in duodenum of these groups. Furthermore, the number of infiltrated lymphocytes into the intestinal epithelium increased significantly in groups administered with 0.25% and 0.5% β-glucan compared with control (Table 6, Fig 2) with highest values in 0.5% β-glucan.

Germinal center areas of spleen in groups administered with 0.25% and 0.5% β-glucan increased ($P < 0.05$) compared with control (Table 7, Fig 3).

The mean fiber cross-sectional area of pectoral muscles and the glycogen areas were significantly improved in groups administered with 0.25% and 0.5% β-glucan (Table 8, Fig 4).

---

### Table 5. Biochemical parameters of rabbits as affected by breed and β-glucan administration.

| Items                      | TP   | Albumin | Globulin | ALT  | AST  | Uric acid | Urea  | Creatinine | A/G Ratio |
|----------------------------|------|---------|----------|------|------|-----------|-------|------------|-----------|
| **Breed**                  |      |         |          |      |      |           |       |            |           |
| NZW                        | 6.03 | 3.42    | 2.60     | 25.15| 13.36| 2.69 *    | 24.59 | 0.84       | 1.39      |
| APRI                       | 6.27 | 3.55    | 2.72     | 22.39| 11.71| 3.02 *    | 27.80 | 0.86       | 1.52      |
| SEM                        | 0.22 | 0.13    | 0.17     | 1.03 | 0.69 | 0.05      | 1.4   | 0.02       | 0.11      |
| $P$ value                  | 0.58 | 0.64    | 0.73     | 0.19 | 0.24 | 0.004     | 0.07  | 0.53       | 0.57      |
| **β-glucan treatment**     |      |         |          |      |      |           |       |            |           |
| βG0.25                     | 6.05 | 3.29    | 2.73     | 20.59| 12.61| 3.04 *    | 29.59 | 0.85       | 1.32      |
| βG0.5                      | 6.34 | 3.44    | 2.90     | 25.91| 11.38| 3.04 *    | 24.62 | 0.86       | 1.31      |
| **Control**                |      |         |          |      |      |           |       |            |           |
| NZW                        | 6.08 | 3.72    | 2.35     | 24.82| 13.61| 2.49 *    | 27.38 | 0.84       | 1.76      |
| APRI                       | 6.08 | 3.72    | 2.35     | 24.82| 13.61| 2.49 *    | 27.38 | 0.84       | 1.76      |
| SEM                        | 0.22 | 0.13    | 0.17     | 1.03 | 0.69 | 0.05      | 1.4   | 0.02       | 0.11      |
| $P$ value                  | 0.82 | 0.43    | 0.42     | 0.11 | 0.44 | 0.001     | 0.36  | 0.92       | 0.20      |
| Breed x treatment interactions |      |         |          |      |      |           |       |            |           |
| NZW                        | 5.92 | 3.11    | 2.23     | 20.25| 15.14| 2.28 *    | 25.77 | 0.83       | 1.74      |
| βG0.25                     | 5.93 | 3.69    | 2.80     | 21.09| 11.97| 2.96 *    | 28.23 | 0.86       | 1.15      |
| βG0.5                      | 6.24 | 3.47    | 2.78     | 34.11| 12.97| 2.84 *    | 19.78 | 0.84       | 1.30      |
| APRI                       | 6.24 | 3.42    | 2.48     | 29.39| 12.07| 2.70 *    | 28.99 | 0.88       | 1.78      |
| βG0.25                     | 6.13 | 3.46    | 2.66     | 20.09| 13.26| 3.12 *    | 30.94 | 0.86       | 1.48      |
| βG0.5                      | 6.45 | 3.76    | 3.02     | 17.71| 9.79 | 3.23 *    | 29.45 | 0.84       | 1.31      |
| SEM                        | 0.22 | 0.13    | 0.17     | 1.03 | 0.69 | 0.05      | 1.4   | 0.02       | 0.11      |
| $P$ value                  | 0.98 | 0.79    | 0.81     | 0.001| 0.40 | 0.001     | 0.27  | 0.954      | 0.54      |

Means within each column for each division with no common superscript letters are significantly different ($P < 0.05$).

SEM = standard error of the mean.

https://doi.org/10.1371/journal.pone.0234076.t005

---
Gene expression assessment

In comparison with the control group, the expressions of intestinal interleukin-18 (IL-18) (Fig 5C) in NZW rabbits administered with 0.25 and 0.5 β-glucan were substantially upregulated. However, in separate treatment groups, there is no important impact on the expression of intestinal IL-4, IL-10, and interferon-γ (IFN-γ) (Fig 1A, 1B and 1D). Rabbits in NZW+βG0.25 and NZW+βG0.5 groups displayed significant upregulations (P < 0.01) in the expression of intestinal superoxide dismutase 1 (SOD1) (Fig 1E) in relation to the control group. In addition, 0.5 β-glucan treated group demonstrated significant upregulation (P < 0.001) of expression of intestinal glutathione peroxidase 1 (GPx1) (Fig 5F) compared with the other group. In addition, the NZW+βG0.5 group showed significant increases (P < 0.05) in intestinal occludin expression compared with the other groups (Fig 5G). NZW rabbit’s mRNA expression of splenic IL-1β, IL-6, and inducible nitric oxide synthase (iNOS) shows no important distinction compared with control (Fig 6A, 6B and 6C).

In APRI breed both 0.25 and 0.5 β-glucan treated groups showed no significant effect on expression levels of intestinal IL-4, IL-6, IL-18, and IFN-γ genes (Fig 7A, 7B, 7C and 7D) as well as splenic IL-1β, IL-6, and iNOS (Fig 4). However, in comparison with control, 0.5 β-glucan treated group showed significant upregulation (P < 0.001) of both SOD1 (Fig 3E) and GPx1 (Fig 7F) mRNA expression, while 0.25 β-glucan treated group showed significant increases (P < 0.05) in GPx1 expression. The 0.5 β-glucan treated group showed significant increases (P < 0.05) in intestinal occludin expression in comparison with control (Fig 7G).

Splenic mRNA expression of IL-1β, IL-6, and iNOS revealed no significant changes in comparison with control APRI rabbits (Fig 8A, 8B and 8C).

Table 6. Histomorphometric changes of rabbits’ duodenum as affected by breed and β-glucan administration (μm).

| Items                  | Villus height | Villus width | Crypt depth | VH/CD | No. of lymphocytes/villi |
|------------------------|---------------|--------------|-------------|-------|-------------------------|
| Breed                  |               |              |             |       |                         |
| NZW                    | 877.89 a      | 118.51 a     | 105.78      | 9.02  | 95.778                  |
| APRI                   | 762.83 b      | 93.39 b      | 110.98      | 7.31  | 99.556                  |
| SEM                    | 18.06         | 3.78         | 5.17        | 0.454 | 1.155                   |
| P value                | 0.004         | 0.002        | 0.647       | 0.072 | 0.128                   |
| β-glucan treatment     |               |              |             |       |                         |
| βG0.25                 | 785.03 b      | 106.60       | 104.25      | 8.29  | 89.500 b                |
| βG0.5                  | 930.89 a      | 106.44       | 111.38      | 8.94  | 126.00 a                |
| Control                | 745.15 b      | 103.30       | 109.52      | 7.25  | 77.500 c                |
| SEM                    | 18.06         | 3.78         | 5.17        | 0.454 | 1.155                   |
| P value                | 0.001         | 0.925        | 0.863       | 0.329 | 0.001                   |
| Breed × treatment interactions |           |              |             |       |                         |
| NZW Control            | 801.19 bcd    | 121.36 a     | 112.16      | 7.57  | 73.333 c                |
| βG0.25                 | 847.94 bc     | 127.29 a     | 100.07      | 9.60  | 88.333 b                |
| βG0.5                  | 984.52 a      | 106.86 ab    | 105.11      | 9.86  | 125.667 a               |
| APRI Control           | 689.11 cd     | 85.23 b      | 106.87      | 6.93  | 81.667 bc               |
| βG0.25                 | 722.11 ad     | 85.92 b      | 108.42      | 6.98  | 90.667 b                |
| βG0.5                  | 877.27 ab     | 106.01 ab    | 117.65      | 8.02  | 126.333 a               |
| SEM                    | 18.06         | 3.78         | 5.17        | 0.454 | 1.155                   |
| P value                | 0.001         | 0.015        | 0.949       | 0.296 | 0.001                   |

Means within each column for each division with no common superscript letters are significantly different (P < 0.05).

VH/CD = Villus height/Crypt depth

https://doi.org/10.1371/journal.pone.0234076.t006
Discussion

Dietary β-glucan administration brought some improvements in animal development and health status [24–26]. Also, β-glucan is considered as an alternative to antibiotics and improves the survival and performance of broilers [27]. In the current study, β-glucan oral administration improved growth performance of NZW and APRI rabbits. Increased efficiency by the nutritional supplement of yeast β-glucan in growing rabbits can lead to increased digestibility and absorption of feedstuffs [28,29]. In addition, improved intestinal health was revealed to increase the villus height, reflecting improved growth efficiency. [30]. In agreement with the current study, Shehata et al [29], Ezema and Eze [31], Bhatt et al [32], and El-Badawi et al [33] found enhancement in BWG and FCR of rabbits administrated with S. cerevisiae and probiotic.

Fig 1. Light micrographs of duodenum revealing the effect of different doses of β glucan; control, 0.25 g β glucan (βG0.25), 0.5 g β glucan (βG0.5) on the two rabbit species; New Zealand White (NZW) and APRI rabbits were represented in (A to C) and from (D to F), respectively. The micrographs showing the increasing in the villi height (VH) from (A to C) and from (D to F). Villi (arrowheads), Brunner’s gland (arrows). PAS stain. Scale bar is 400 μm.
https://doi.org/10.1371/journal.pone.0234076.g001
Some studies support the idea of using prebiotics for increasing the length of the intestinal villus and enhancing immunity [34,35]. Also, this leads to better nutrient absorption, and consequently increases body weight [36]. The enhanced villi height to crypt depth, which would...
permit higher nutrient intake, may explain enhanced growth efficiency as a response to β-glucans [10,37] and improved intestinal barrier function [38]. Seyidoglu and Peker [39] demonstrated significant increases in thickness of the mucosa, villus heights, crypt depths, and gland depths in rabbits fed diets administrated with yeast that contains β-glucan. A high V/C ratio indicates sufficiently matured and functionally active epithelial cells [40]. In this study, the longest villi values for duodenum were recorded for 0.5 β-glucan, followed by 0.25 β-glucan for both breeds and reflects the absorptive capacity of the intestine.

The present study detected increases of white pulp areas by 0.25 G β-glucan, which reflect on the increase of rabbit immunity. Increased fatty acid utilization due to β-glucan treatment in high fat diet fed mice has been stated by Miyamoto et al [41] and led to a decreased glycogen depletion rate and increased glycogen accumulation in the liver and muscle [42]. Xu et al [43] observed a significant increase in non-esterified fatty acids’ concentration in β-glucan feeding rats, which indicates that β-glucan improves muscle quality due to the increased availability of glycogen. Interestingly, our results showed the significant increase of fiber cross-sectional area of pectoral muscles in 0.5 β-glucan groups. Moreover, glycogen areas were higher in 0.5 G β-glucan of NZW breed and 0.25 β-glucan of APRI breed owing to the higher proportion of high glycogen muscle fibers compared to low glycogen muscle fibers. Therefore, β-glucan could improve the meat quality of rabbits.

Concerning the biochemical findings, ElSawy et al [24] reported that oral administration of yeast β-glucan did not alter serum protein, albumin, and globulin of chicks in comparison with control chicks. Belhassen et al [44] reported that dietary administration of S. cerevisiae did not alter blood parameters of growing rabbits. The increased uric acid levels in β-glucan-administrated groups may be due to enhancement of purine metabolism and not due to

### Table 7. Averages of total splenic white pulp areas/ 3 mm².

| Items                  | Total splenic white pulp area |
|------------------------|-------------------------------|
| Breed                  |                               |
| NZW                    | 44811.79 a                    |
| APRI                   | 30146.46 b                    |
| SEM                    | 1869.503                      |
| P value                | 0.002                         |
| β-glucan treatment     |                               |
| βG0.25                 | 45430.136 b                   |
| βG0.5                  | 40873.803 b                   |
| Control                | 26133.440 b                   |
| SEM                    | 1869.503                      |
| P value                | 0.003                         |
| Breed × treatment interactions |                   |
| NZW                    |                               |
| Control                | 34570.902 b                   |
| G0.25                  | 60290.571 b                   |
| βG0.5                  | 39573.901 b                   |
| APRI                   |                               |
| Control                | 17695.979 b                   |
| βG0.25                 | 30569.700 bbc                 |
| βG0.5                  | 42173.704 b                   |
| SEM                    | 1869.503                      |
| P value                | 0.001                         |

Means within each column for each division with no common superscript letters are significantly different (P < 0.05).

https://doi.org/10.1371/journal.pone.0234076.t007
increased kidney function because urea and creatinine levels did not have any changes compared with control.

IL-18 operates to induce a Th1-mediated reaction after exposure to a pathogen in association with IL-12 [45]. An initial increase in intestinal IL-18 gene expression was observed in an unchallenged study on day 7 due to dietary β-glucan, followed by a downregulation on day 14. These findings were consistent with our research, which revealed that the expression of intestinal IL-18 in NZW rabbit treated with 0.25 and 0.5 β-glucan was considerably upregulated with respect to the control group. IL-18 is an IL-1 family cytokine that has been proposed to promote barrier function in the intestine that improved the gut health against pathogens [46]. In a subsequent research, the expression of IL-18 in birds' jejunums fed the β-glucan diet was improved [47].
Our outcome shows that there is no important impact on the expression levels of intestinal interleukin-4 (IL-4), IL-10, and splenic IL-6 in separate treatment groups and that the amount of expression of splenic IL1β, IL-6, and inducible nitric oxide synthase (iNOS) in NZ and APRI rabbits shows no important distinction in the control group. The proinflammatory cytokine IL-1 secretion is enhanced by β-glucan [48]. Contradictory information was gathered in mammals where concentrations of IL-6 and TNF-α in β-glucan-fed pigs subjected to lipopolysaccharide decreased relative to their controls [49]. Similar outcomes were noted where intramuscular injection of β-glucan in Wistar rats blocked TNF-α, IL-1β, and IL-6 elevations observed in the control group following sepsis-induced lung injury [50].

> When exposed to antigens or chemotactic agents, macrophages start to build iNOS. This enzyme contributes to the development of nitric oxide that then binds to toxic derivatives with superoxide anions, allowing macrophages to skillfully destroy a few kinds of pathogens [51]. Our outcome showed that there were no changes in the splenic iNOS expression rate of mRNA in both NZW and APRI. Cox et al [52] They found no important variations in the rate of expression of the iNOS gene.

> Cellular GSH is an essential cellular antioxidant molecule that aids in scavenging of radical species or involvement in antioxidant enzyme catalyzed responses such as GPx [53]. Also, SOD is a critical antioxidant enzyme that protects the cells from the harmful effects of superoxide anion radical [54]. Pretreatment with melatonin or β-D-glucan lowered the harm caused by acetaminophen-induced hepatotoxicity by decreasing oxidative pressure and growing antioxidant activity of GPx, SOD, and catalase (CAT), because melatonin or β-glucan are recognized as free-radical scavengers [55].

### Table 8. Mean of muscle fiber cross-sectional area (μm²) and glycogen area/300 μm².

| Items          | Muscle fiber cross-sectional area | Glycogen area |
|----------------|----------------------------------|---------------|
| **Breed**      |                                  |               |
| NZW            | 2330.79 a                        | 728.10 a      |
| APRI           | 2048.90 b                        | 454.11 b      |
| SEM            | 62.56                            | 54.06         |
| **P value**    |                                  |               |
|                 | 0.02                             | 0.02          |
| **β-glucan treatment** |                              |               |
| βG₀.25 2257.75 b |                                  | 762.97 a      |
| βG₀.5          | 2672.75 a                        | 837.75 a      |
| Control        | 1639.53 a                        | 172.88 b      |
| SEM            | 62.56                            | 54.06         |
| **P value**    |                                  |               |
|                 | 0.01                             | 0.01          |
| **Breed × treatment interactions** |                              |               |
| NZW            |                                  |               |
| Control        | 1775.40 c                        | 108.45 c      |
| βG₀.25         | 2502.11 a                        | 1099.70 a     |
| βG₀.5          | 2714.86 a                        | 976.14 a      |
| APRI           |                                  |               |
| Control        | 1503.65 c                        | 237.30 c      |
| βG₀.25         | 2013.40 b                        | 425.64 bc     |
| βG₀.5          | 2629.66 a                        | 699.37 ab     |
| SEM            | 62.56                            | 54.06         |
| **P value**    |                                  |               |
|                 | 0.01                             | 0.01          |

Means within each column for each division with no common superscript letters are significantly different (P < 0.05).

https://doi.org/10.1371/journal.pone.0234076.t008

PLOS ONE | https://doi.org/10.1371/journal.pone.0234076 June 10, 2020 14 / 24
Tight junctions consist of at least three types of transmembrane proteins: occludin, claudins, and molecules of junctional adhesion. Occludin and the family of claudins are the most significant elements of epithelial barrier function in the intestine [56]. Results also indicated that β-glucan upregulated the expression of the intestinal occludin mRNA, especially at 0.5 β-glucan.
Fig 5. RT-PCR validation of the intestinal (A) interleukin-4 (IL-4), (B) interleukin-10 (IL-10), (C) interleukin-18 (IL-18), (D) interferon-γ (IFN-γ), (E) superoxide dismutase 1 (SOD1), (F) glutathione peroxidase 1 (GPx1), and (G) occludin genes in NZW rabbits. *P < 0.05, **P < 0.01 and ***P < 0.001 vs. control. +++P < 0.001 vs. NZW+βG0.25. Statistical analysis was performed using one-way ANOVA and Tukey’s post hoc test for multiple comparisons.

https://doi.org/10.1371/journal.pone.0234076.g005
Fig 6. RT-PCR validation of the splenic (A) interleukin-1 beta (IL1β), (B) interleukin-6 (IL-6), and (C) inducible nitric oxide synthase (iNOS) genes in NZW rabbits. Statistical analysis was performed using one-way ANOVA and Tukey’s post hoc test for multiple comparisons.

https://doi.org/10.1371/journal.pone.0234076.g006
Fig 7. RT-PCR validation of the intestinal (A) interleukin-4 (IL-4), (B) interleukin-10 (IL-10), (C) interleukin-18 (IL-18), (D) interferon-γ (IFN-γ), (E) superoxide dismutase 1 (SOD1), (F) glutathione peroxidase 1 (GPx1), and (G) occludin genes in APRI rabbits. *P < 0.05 and **P < 0.001 vs. control. ***P < 0.001 vs. APRI+βG0.25. Statistical analysis was performed using one-way ANOVA and Tukey’s post hoc test for multiple comparisons.

https://doi.org/10.1371/journal.pone.0234076.g007
β-glucan supplementation improves gut environment in rabbits

(A) Splenic IL-1β
(B) Splenic IL-6
(C) Splenic iNOS

Expression fold change
Conclusion

Dietary immunomodulators such as yeast β-glucan attract considerable attention because they promote indirect development by enhancing immunocompetence in food animals. Here, β-glucan significantly improved villi dimensions, splenic lymphoid diameter, muscular fiber diameter, and muscular glycogen areas. Regarding the breed type, NZW rabbits showed better growth performance than APRI rabbits as represented in the final body weight total daily gain, feed consumption, and total feed conversion ratio. However, carcass traits did not show any significant differences in both rabbit breeds. Oral administration of β-glucan in rabbits will minimize the use of antibiotics, thereby reducing the possible occurrence of drug resistance in bacteria.

Supporting information

S1 File. Raw data of RT-PCR in NZW. (PZF)

S2 File. Raw data of RT-PCR in APRI. (PZF)

Author Contributions

Conceptualization: Mahmoud M. Abo Ghanima, Ayman H. Abd El-Aziz, Ahmed E. Noreldin, Mustafa S. Atta, Ali H. El-Far.

Data curation: Mahmoud M. Abo Ghanima, Ayman H. Abd El-Aziz, Ahmed E. Noreldin, Mustafa S. Atta, Shaker A. Mousa, Ali H. El-Far.

Formal analysis: Mahmoud M. Abo Ghanima, Ahmed E. Noreldin, Mustafa S. Atta, Ali H. El-Far.

Funding acquisition: Mahmoud M. Abo Ghanima, Ayman H. Abd El-Aziz, Mustafa S. Atta, Ali H. El-Far.

Investigation: Mahmoud M. Abo Ghanima, Ahmed E. Noreldin, Mustafa S. Atta, Ali H. El-Far.

Methodology: Mahmoud M. Abo Ghanima, Ayman H. Abd El-Aziz, Ahmed E. Noreldin, Mustafa S. Atta, Ali H. El-Far.

Project administration: Mahmoud M. Abo Ghanima, Ayman H. Abd El-Aziz.

Resources: Ayman H. Abd El-Aziz, Ahmed E. Noreldin.

Software: Mahmoud M. Abo Ghanima, Ahmed E. Noreldin, Mustafa S. Atta, Shaker A. Mousa, Ali H. El-Far.

Supervision: Shaker A. Mousa, Ali H. El-Far.

β-glucan upregulated intestinal occludin mRNA has anti-inflammatory and antioxidant characteristics [57].
Validation: Ayman H. Abd El-Aziz, Ahmed E. Noreldin, Mustafa S. Atta, Shaker A. Mousa, Ali H. El-Far.

Visualization: Mahmoud M. Abo Ghanima, Ayman H. Abd El-Aziz, Ahmed E. Noreldin, Mustafa S. Atta, Shaker A. Mousa, Ali H. El-Far.

Writing – original draft: Mahmoud M. Abo Ghanima, Ayman H. Abd El-Aziz, Ahmed E. Noreldin, Mustafa S. Atta, Shaker A. Mousa, Ali H. El-Far.

Writing – review & editing: Mahmoud M. Abo Ghanima, Ayman H. Abd El-Aziz, Ahmed E. Noreldin, Mustafa S. Atta, Shaker A. Mousa, Ali H. El-Far.

References
1. Attia YA, Al-Hanoun A, Bovera F. Effect of different levels of bee pollen on performance and blood profile of New Zealand White bucks and growth performance of their offspring during summer and winter months. J Anim Physiol Anim Nutr (Berl). 2011; 95: 17–26. https://doi.org/10.1111/j.1439-0396.2009.00967.x PMID: 20455966
2. Dalle Zotte A, Szendrö Z. The role of rabbit meat as functional food. Meat Sci. 2011; 88: 319–331. https://doi.org/10.1016/j.meatsci.2011.02.017 PMID: 21392894
3. Ebeid TA, Basyony MM, Dosoky WM, Badry H. Fortification of rabbit diets with vitamin E or selenium affects growth performance, lipid peroxidation, oxidative status and immune response in growing rabbits. Livest Sci. 2013; https://doi.org/10.1016/j.livsci.2013.11.004
4. Petracci M, Bianchi M, Cavani C. Development of Rabbit Meat Products Fortified With n-3 Polysaturated Fatty Acids. Nutrients. Molecular Diversity Preservation International; 2009; 1: 111–118. https://doi.org/10.3390/nu1020111 PMID: 22253971
5. Dalle Zotte A. Perception of rabbit meat quality and major factors influencing the rabbit carcass and meat quality. Livest Prod Sci. Elsevier; 2002; 75: 11–32. https://doi.org/10.1016/S0301-6226(01)00308-6
6. Falcão-e-Cunha L., Castro-Solla L. GC, Maertens L. L, Marounek M. M, Pinheiro V. V, Freire J. J, et al. Alternatives to antibiotic growth promoters in rabbit feeding: a review. World Rabbit Sci. 2010; 15: 127–140. https://doi.org/10.4995/wrs.2007.597
7. Brown GD, Gordon S. Immune recognition of fungal β-glucans. Cell Microbiol. 2005; 7: 471–479. https://doi.org/10.1111/j.1462-5822.2005.00505.x PMID: 15760447
8. Vetvicka V, Dvorak B, Vetvickova J, Richter J, Krizan J, Sima P, et al. Orally administered marine (1→3)-β-glucan Phycarine stimulates both humoral and cellular immunity. Int J Biol Macromol. 2007; 40: 291–298. https://doi.org/10.1016/j.ijbiomac.2006.08.009 PMID: 16978690
9. Crespo H, Guillén H, de Pablo-Maiso L, Gómez-Arrebola C, Rodríguez G, Glaria I, et al. Lentinula edodes β-glucan enriched diet induces pro- and anti-inflammatory macrophages in rabbit. Food Nutr Res. Swedish Nutrition Foundation; 2017; 61: 1412791. https://doi.org/10.1080/16546628.2017.1412791 PMID: 28249921
10. Chen K-L, Weng B-C, Chang M-T, Liao Y-H, Chen T-T, Chu C. Direct Enhancement of the Phagocytic and Bacterial Capability of Abdominal Macrophage of Chicks by -1,3-1,6-Glucan. Poult Sci. 2008; 87: 2242–2249. https://doi.org/10.3382/ps.2008-00147 PMID: 18931174
11. Hahn T-W, Lohakare JD, Lee SL, Moon WK, Chae BJ. Effects of supplementation of β-glucans on growth performance, nutrient digestibility, and immunity in weanling pigs. J Anim Sci. 2006; 84: 1422–1428. https://doi.org/10.2527/2006.8461422x PMID: 16699099
12. Krakowski L, Krzyzanowski J, Wrona Z, Siwicki AK. The effect of nonspecific immunostimulation of pregnant mares with 1,3-1,6 glucan and levamisole on the immunoglobulins levels in colostrum, selected indices of nonspecific cellular and humoral immunity in foals in neonatal and postnatal period. Vet Immunol Immunopathol. 1999; 68: 1–11. Available: http://www.ncbi.nlm.nih.gov/pubmed/10231947 https://doi.org/10.1016/s0165-2427(99)00006-9 PMID: 10231947
13. NRC. Nutrient Requirements of Rabbits. National Academy of Science, Washington, D.C; 1977.
14. De Blas C, Wiseman J. The Nutrition of the Rabbit Edited by. 2010.
15. Lambert WV, Ellis NR, Block WH, Titus HW. The role of nutrition in genetics. Am Res Soc Anim Prod. 1936; 29: 236.
16. Blasco A., Ouhayoun J. J. Harmonization of criteria and terminology in rabbit meat research. Revised proposal. World Rabbit Sci. 2010; 4: 93–99. https://doi.org/10.4995/wrs.1996.278
17. Bancroft J, Layton C. The Hematoxylin and eosin. In: Suvarna S. K, Layton C, Bancroft J. D, editors. Theory Practice of histological techniques., 7th edn. Philadelphia: Churchill Livingstone of El Sevier; Philadelphia: Churchill Livingstone of El Sevier; 2013.

18. Saeed M, Yatoa X, Hassan F, Arain M, Abd El-Hack M, Noreldin A, et al. Influence of Graded Levels of i-Theanine Dietary Supplementation on Growth Performance, Carcass Traits, Meat Quality, Organs Histomorphometry, Blood Chemistry and Immune Response of Broiler Chickens. Int J Mol Sci. 2018; 19: 462. https://doi.org/10.3390/ijms19020462 PMID: 29401695

19. Kiczorowska B, Al-Yasiry ARM, Samoli Saeed M, Yatao X, Hassan F, Arain M, Abd El-Hack M, Noreldin A, et al. Influence of Graded Levels of i-Theanine Dietary Supplementation on Growth Performance, Carcass Traits, Meat Quality, Organs Histomorphometry, Blood Chemistry and Immune Response of Broiler Chickens. Int J Mol Sci. 2018; 19: 462. https://doi.org/10.3390/ijms19020462 PMID: 29401695

20. Heywood JL, McEntee GM, Stickland NC. In ovo neuromuscular stimulation alters the skeletal muscle phenotype of the chick. J Muscle Res Cell Motil. 2005; 26: 49–56. https://doi.org/10.1007/s10974-005-9007-8 PMID: 16088375

21. Prats C, Gomez-Cabello A, Nordby P, Andersen JL, Helge JW, Dela F, et al. An optimized histochemical method to assess skeletal muscle glycogen and lipid stores reveals two metabolically distinct populations of type I muscle fibers. PLoS One. Public Library of Science; 2013; 8: e77774. https://doi.org/10.1371/journal.pone.0077774 PMID: 24204859

22. Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res. 2001; 29: 45e–45. https://doi.org/10.1093/nar/29.9.e45 PMID: 11328886

23. Duncan DB. Multiple Range and Multiple F Tests. Biometr ics. International Biometric Society; 1955; 11: 1. https://doi.org/10.2307/3001478

24. ElSawy A, ElMaddawy Z, BoGhazel H. The Growth Promoting Effect of Beta-glucan in Comparison with Sodium Butyrate in Broiler Chicks. Alexandria J Vet Sci. 2015; 44: 23. https://doi.org/10.5455/ajvs.163992

25. Attia YA, Hamed RS, Abd El-Hamid AE, Al-Harthi MA, Shahba F, Bovera HA. Performance, blood profile, carcass and meat traits and tissue morphology in growing rabbits fed mannanoligosaccharides and zinc-bactracin continuously or intermittently. Anim Sci Pap Reports. Polish Scientific Publishers; 2015; 33: 85–101. Available: http://agro.icm.edu.pl/agro/element/bwmeta1.element.agro-f60df433-4194-4e39-a680-177a76aefdb5

26. Khanna S, Gullati HK, Verma AK, Shag SS, Sharma DP, Kapoor PK. Effect of yeast supplementation and alternative housing systems on performance of rabbits. Haryana Vet. College of Veterinary Sciences. Haryana Agricultural University; 2014; 53: 23–27. Available: https://www.cabdirect.org/cabdirect/abstract/20143401914

27. Moon SH, Lee I, Fang X, Lee HY, Kim J, Ahn DU. Effect of Dietary Beta-Glucan on the Performance of Broilers and the Quality of Broiler Breast Meat. Asian-Australasian J Anim Sci. Asian-Australasian Association of Animal Production Societies (AAAP); 2016; 29: 384. https://doi.org/10.5713/AJAS.15.0141 PMID: 26950870

28. Resta-Lereit S, Barrett KE. Live probiotics protect intestinal epithelial cells from the effects of infection with enteroinvasive Escherichia coli (EIEC). Gut. 2003; 52: 988–997. https://doi.org/10.1136/gut.52.7.988 PMID: 12801956

29. Shehata SA, Mahrose KM, Ismail EI. Effect of amino yeast addition on growth performance, digestion, carcass traits and economical efficiency of growing rabbit. Egypt J Nutr Feed. 2012; 15: 75–80.

30. Zhang AW, Lee BD, Lee SK, Lee KW, An GH, Song KB, et al. Effects of yeast (Saccharomyces cerevisiae) cell components on growth performance, meat quality, and ileal mucosa development of broiler chicks. Poult Sci. 2005; 84: 1015–1021. https://doi.org/10.1093/ps/84.7.1015 PMID: 16050118

31. Ezema C, Eze DC. Determination of the effect of probiotic (Saccharomyces cerevisiae) on growth performance and hematological parameters of rabbits. Comp Clin Path. Springer-Verlag; 2012; 21: 73–76. https://doi.org/10.1007/s00580-010-1066-6

32. Bhatt RS, Agrawal AR, Sahoo A. Effect of probiotic supplementation on growth performance, nutrient utilization and carcass characteristics of growing Chinchilla rabbits. J Appl Anim Res. Taylor & Francis; 2017; 45: 304–309. https://doi.org/10.1080/09712119.2016.1174126

33. El-Badawi AY. Growth performance of male NZW rabbits fed diets supplemented with beneficial bacteria or live yeast. Agric Eng Int CIGR J. 2018; 19: 220–226.

34. Abd El-Hack ME, Samak DH, Noreldin AE, El-Naggar K, Abdo M. Probiotics and plant-derived compounds as eco-friendly agents to inhibit microbial toxins in poultry feed: a comprehensive review. Environ Sci Pollut Res. 2018; 25: 31971–31986. https://doi.org/10.1007/s11356-018-3197-2 PMID: 30229484

35. Teng P-Y, Kim WK. Review: Roles of Prebiotics in Intestinal Ecosystem of Broilers. Front Vet Sci. Frontiers; 2018; 5: 245. https://doi.org/10.3389/fvets.2018.00245 PMID: 30425993

β-glucan supplementation improves gut environment in rabbits
36. Chen TC. Effect of adding chicory fructans in feed on broiler growth performance, serum cholesterol and intestinal length. Int J Poult Sci. Citeseer; 2003.

37. Tsukada C, Yokoyama H, Miyai C, Ishimoto Y, Kawamura H, Abo T. Immunopotentiation of intraepithelial lymphocytes in the intestine by oral administrations of beta-glucan. Cell Immunol. 2003; 221: 1–5. https://doi.org/10.1016/s0008-8749(03)00061-3 PMID: 12742376

38. Shao Y, Guo Y, Wang Z: -1,3/1,6-Glucan alleviated intestinal mucosal barrier impairment of broiler chickens challenged with Salmonella enterica serovar Typhimurium. Poult Sci. 2013; 92: 1764–1773. https://doi.org/10.3382/ps.2013-03029 PMID: 23776263

39. Seyidoglu N, Peker S. Effects of different doses of probiotic yeast Saccharomyces cerevisiae on the duodenal mucosa in rabbits. Indian J Anim Res. 2015; 49: 602–606.

40. Tian X, Shao Y, Wang Z, Guo Y. Effects of dietary beta-glucans on performance, gut morphology, intestinal Clostridium perfringens population and immune response of broiler chickens challenged with necrotic enteritis. Anim Feed Sci Technol. Elsevier; 2016; 215: 144–155. https://doi.org/10.1016/j.anipts.2016.03.009

41. Miyamoto J, Watanabe K, Taia S, Kasubuchi M, Li X, Irie J, et al. Barley beta-glucan improves metabolic condition via short-chain fatty acids produced by gut microbial fermentation in high fat diet fed mice. Nerurkar P V, editor. PLoS One. 2018; 13: e0196579. https://doi.org/10.1371/journal.pone.0196579 PMID: 29698465

42. Azevedo JL, Linderman JK, Lehman SL, Brooks GA. Training decreases muscle glycogen turnover during exercise. Eur J Appl Physiol Occup Physiol. 1998; https://doi.org/10.1007/s0042100050449 PMID: 9840401

43. Xu C, Lv J, Lo YM, Cui SW, Hu X, Fan M. Effects of oat beta-glucan on endurance exercise and its anti-fatigue properties in trained rats. Carbohydr Polym. 2013; 92: 1159–1165. https://doi.org/10.1016/j.carbpol.2012.02.023 PMID: 23399141

44. Belhassen T, Bonai A, Gerencser Z, Matics Z, Tuboly T, Bergaoui R, et al. Effect of diet supplementation with live yeast Saccharomyces cerevisiae on growth performance, caecal ecosystem and health of growing rabbits. World Rabbit Sci. 2016; 24: 191. https://doi.org/10.4995/wrs.2016.3991

45. Hong YH, Lillehoj HS, Lee SH, Dalloul RA, Lillehoj EP. Analysis of chicken cytokine and chemokine gene expression following Eimeria acervulina and Eimeria tenella infections. Vet Immunol Immunopathol. 2006; 114: 209–223. https://doi.org/10.1016/j.vetimm.2005.11.007 PMID: 16996141

46. Harrison OJ, Srinivasan N, Pott J, Schiering C, Krausgruber T, Ilott NE, et al. Epithelial-derived IL-18 regulates Th17 cell differentiation and Foxp3+ Treg cell function in the intestine. Mucosal Immunol. Nature Publishing Group; 2015; 8: 1226–1236. https://doi.org/10.1038/mi.2015.13 PMID: 25736457

47. Cox CM, Summers LH, Kim S, McElroy AP, Bedford MR, Dalloul RA. Immune responses to dietary -glucan in broiler chicks during an Eimeria challenge. Poult Sci. 2010; 89: 2597–2607. https://doi.org/10.3382/ps.2010-00987 PMID: 21076097

48. Guo Y, Ali RA, Qureshi MA. The influence of beta-glucan on immune responses in broiler chicks. Immunopharmacol Immunotoxicol. 2003; 25: 461–72. Available: http://www.ncbi.nlm.nih.gov/pubmed/19180808 https://doi.org/10.1080/1740390500247832 PMID: 19180808

49. Li J, Xing J, Li D, Wang X, Zhao L, Lv S, et al. Effects of beta-glucan extracted from Saccharomyces cerevisiae on humoral and cellular immunity in weaned piglets. Arch Anim Nutr. 2005; 59: 303–312. https://doi.org/10.1080/17450390500247832 PMID: 16320779

50. Bedirli A, Kerem M, Pasaoglu H, Akurek N, Tezcaner T, Elbeg S, et al. Beta-glucan attenuates inflammatory cytokine release and prevents acute lung injury in an experimental model of sepsis. Shock. 2007; 27: 397–401. https://doi.org/10.1097/01.shk.0000245030.24235.1f PMID: 17144422

51. Tizard IR. Veterinary Immunology. Elsevier Health Sciences; 2018; 54: 287–293. https://doi.org/10.5455/medscience.2016.05.8429
56. Fanning AS, Jameson BJ, Jesaitis LA, Anderson JM. The tight junction protein ZO-1 establishes a link between the transmembrane protein occludin and the actin cytoskeleton. J Biol Chem. 1998; 273: 29745–29753. https://doi.org/10.1074/jbc.273.45.29745 PMID: 9792688

57. Krizková L, Duracková Z, Sandula J, Slamenová D, Sasinková V, Sivonová M, et al. Fungal beta-(1–3)-D-glucan derivatives exhibit high antioxidative and antimutagenic activity in vitro. Anticancer Res. 2003; 23: 2751–6. Available: http://www.ncbi.nlm.nih.gov/pubmed/12894570 PMID: 12894570